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Bevilacqua et al.

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(45) **Date of Patent: Sep. 20, 2016**

(54) **COMPOSITIONS AND USES OF ANTIMICROBIAL MATERIALS WITH TISSUE-COMPATIBLE PROPERTIES**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 32 days.

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Related U.S. Application Data

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(51) **Int. Cl.**

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A61K 38/02	(2006.01)
A61K 38/16	(2006.01)
A61K 9/06	(2006.01)
A61L 15/42	(2006.01)
A61L 15/46	(2006.01)
A61L 24/00	(2006.01)
A61K 31/715	(2006.01)
A61K 31/765	(2006.01)
A01N 37/46	(2006.01)
A01N 37/44	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 38/02*** (2013.01); ***A01N 37/44*** (2013.01); ***A01N 37/46*** (2013.01); ***A61K 9/06*** (2013.01); ***A61K 31/715*** (2013.01); ***A61K 31/765*** (2013.01); ***A61K 38/16*** (2013.01); ***A61K 45/06*** (2013.01); ***A61L 15/42*** (2013.01); ***A61L 15/46*** (2013.01); ***A61L 24/001*** (2013.01); ***A61L 24/0015*** (2013.01); ***A61L 24/0036*** (2013.01); *A61L 2400/04* (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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Vidaza (azacitidine for injection) Product Label, Approved 2004; Revised: Aug. 2011, Reference ID: 3002164.

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(57) **ABSTRACT**

Compositions comprising a mixture of an antimicrobial cationic polypeptide and a second pharmaceutically-acceptable polymer are disclosed, as well as methods and uses thereof for the treatment and prevention of infections that occur when our natural barriers of defense are broken.

18 Claims, 30 Drawing Sheets

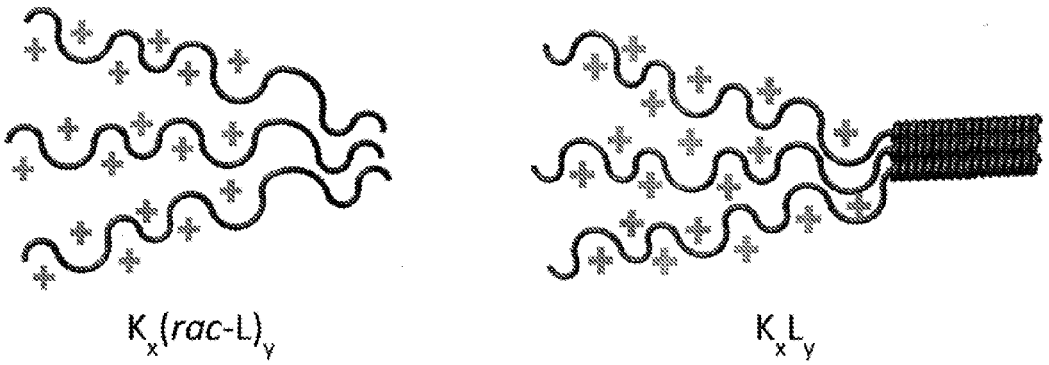


FIG. 1

FIG. 2A

$[K_{65}(rac\text{-}L)_{15}\text{-}RAN]\text{-}(rac\text{-}L)_{20}$	$K_{100}(rac\text{-}L)_{30}$	$K_{120}(rac\text{-}L)_{10}$	$K_{130}L_{40}\text{-}RAN$
$K_{60}(rac\text{-}L)_{20}$	$K_{100}L_{40}$	$K_{130}L_{20}$	$K_{130}L_{60}$
K_{100}	$K_{100}(rac\text{-}L)_{40}$	$K_{130}(rac\text{-}L)_{20}$	$K_{130}(rac\text{-}L)_{60}$
$K_{100}L_{20}$	$K_{100}L_{40}\text{-}RAN$	$K_{130}L_{30}$	$K_{150}L_{30}$
$K_{102}(rac\text{-}L)_{20}$	$K_{100}L_{50}$	$K_{130}(rac\text{-}L)_{30}$	$K_{160}(rac\text{-}L)_{20}$
$K_{100}(rac\text{-}L)_{20}\text{-}RAN$	$K_{100}L_{60}$	$K_{130}L_{40}$	$K_{180}(rac\text{-}L)_{20}$
$K_{100}L_{30}$	$K_{100}(rac\text{-}L)_{60}$	$K_{130}(rac\text{-}L)_{40}$	$K_{200}L_{50}$

FIG. 2B

K_{55}	$K_{180}L_{54}$	$K_{360}L_{72}$	$E_{324}L_{36}$
$K_{99}L_{36}$	$K_{180}(rac\text{-}L)_{54}$	$K_{360}(rac\text{-}L)_{72}$	$E_{360}L_{36}$
$K_{99}(rac\text{-}L)_{36}$	$K_{324}L_{36}$	E_{180}	$E_{360}(rac\text{-}L)_{36}$
$K_{99}(rac\text{-}L)_{36}\text{-}RAN$	K_{360}	$E_{180}L_{18}$	$E_{360}L_{54}$
K_{180}	$K_{360}L_{36}$	$E_{180}(rac\text{-}L)_{18}$	$E_{360}(rac\text{-}L)_{54}$
$K_{180}L_{18}$	$K_{360}(rac\text{-}L)_{36}$	$E_{180}L_{36}$	$E_{360}L_{72}$
$K_{180}(rac\text{-}L)_{18}$	$K_{360}L_{36}\text{-}RAN$	$E_{180}(rac\text{-}L)_{36}$	$E_{360}(rac\text{-}L)_{72}$
$K_{180}L_{36}$	$K_{360}L_{54}$	$E_{180}L_{54}$	
$K_{180}(rac\text{-}L)_{36}$	$K_{360}(rac\text{-}L)_{54}$	$E_{180}(rac\text{-}L)_{54}$	

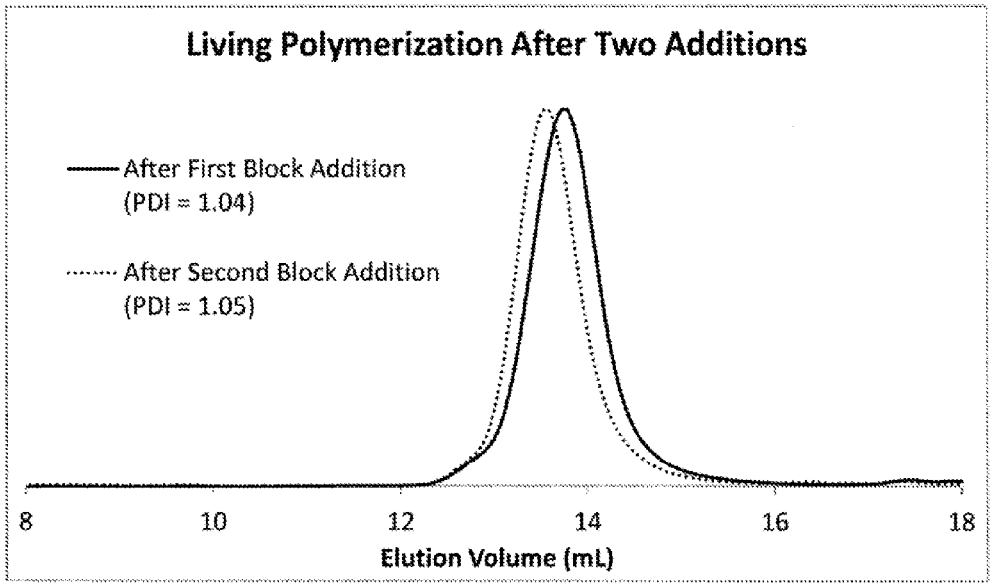

Living Polymerization After Two Additions
After First Block Addition
(PDI = 1.04)
After Second Block Addition
(PDI = 1.05)
8
10
12
14
16
18
Elution Volume (mL)

FIG. 3

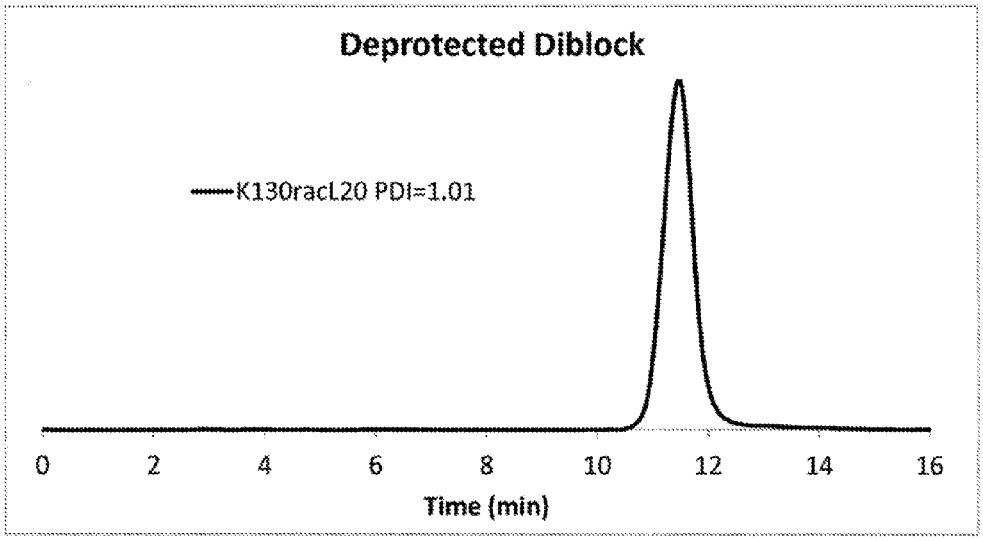

Deprotected Diblock
K130racL20 PDI=1.01
0
2
4
6
8
10
12
14
16
Time (min)

FIG. 4

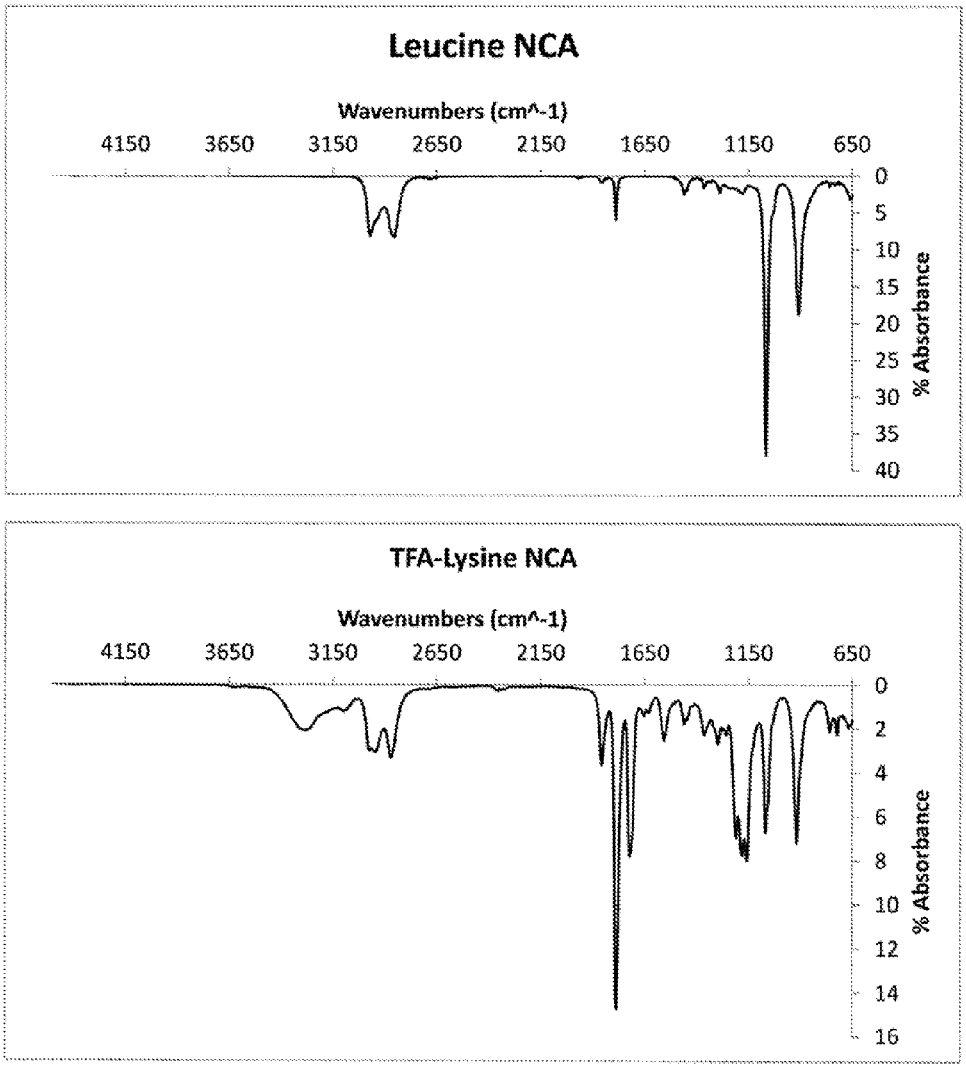

Leucine NCA
Wavenumbers (cm^-1)
4150
3650
3150
2650
2150
1650
1150
650
0
5
10
15
20
25
30
35
40
% Absorbance
TFA-Lysine NCA
Wavenumbers (cm^-1)
4150
3650
3150
2650
2150
1650
1150
650
0
2
4
6
8
10
12
14
16
% Absorbance

FIG. 5

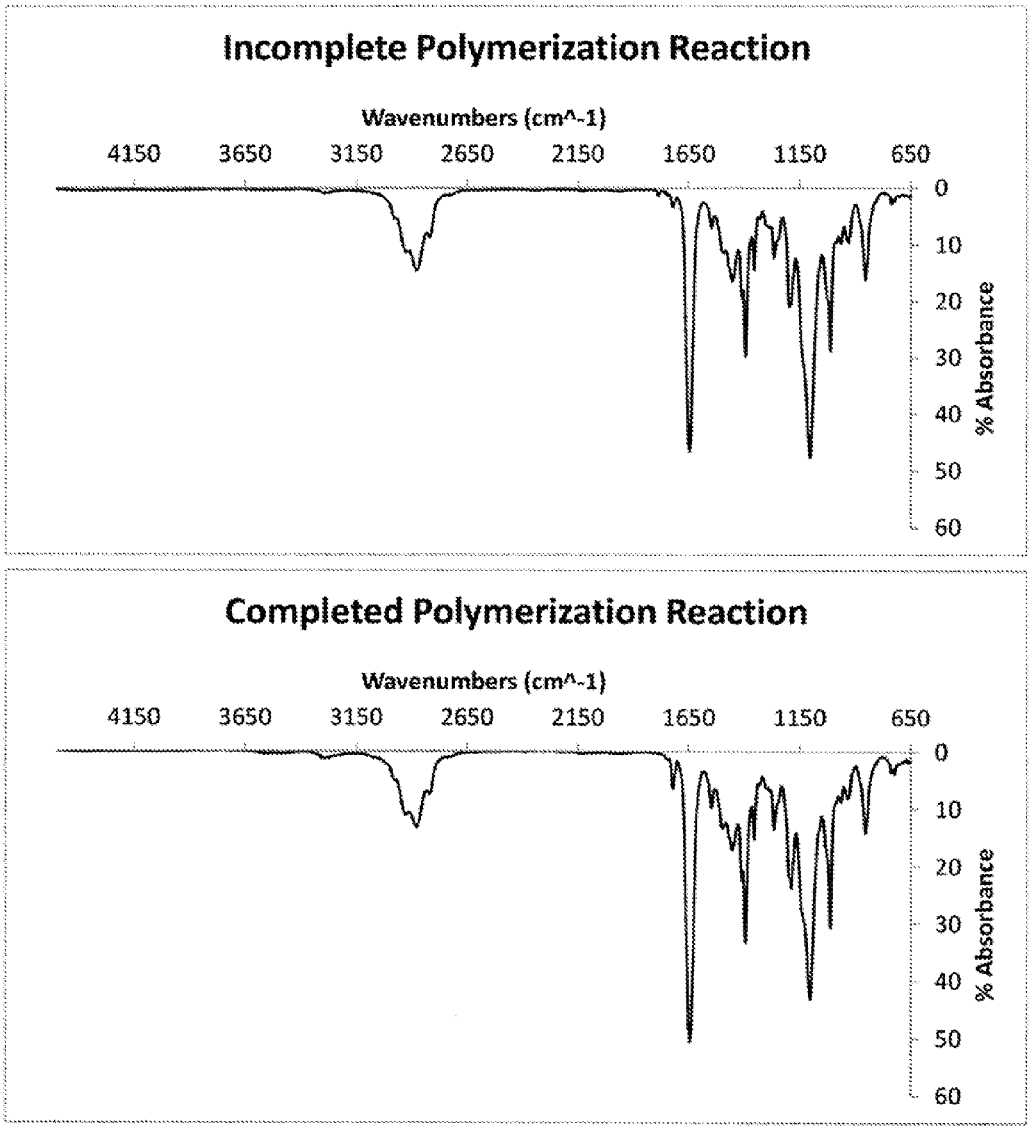

Incomplete Polymerization Reaction
Wavenumbers (cm^-1)
4150
3650
3150
2650
2150
1650
1150
650
0
10
20
30
40
50
60
% Absorbance
Completed Polymerization Reaction
Wavenumbers (cm^-1)
4150
3650
3150
2650
2150
1650
1150
650
0
10
20
30
40
50
60
% Absorbance

FIG. 6

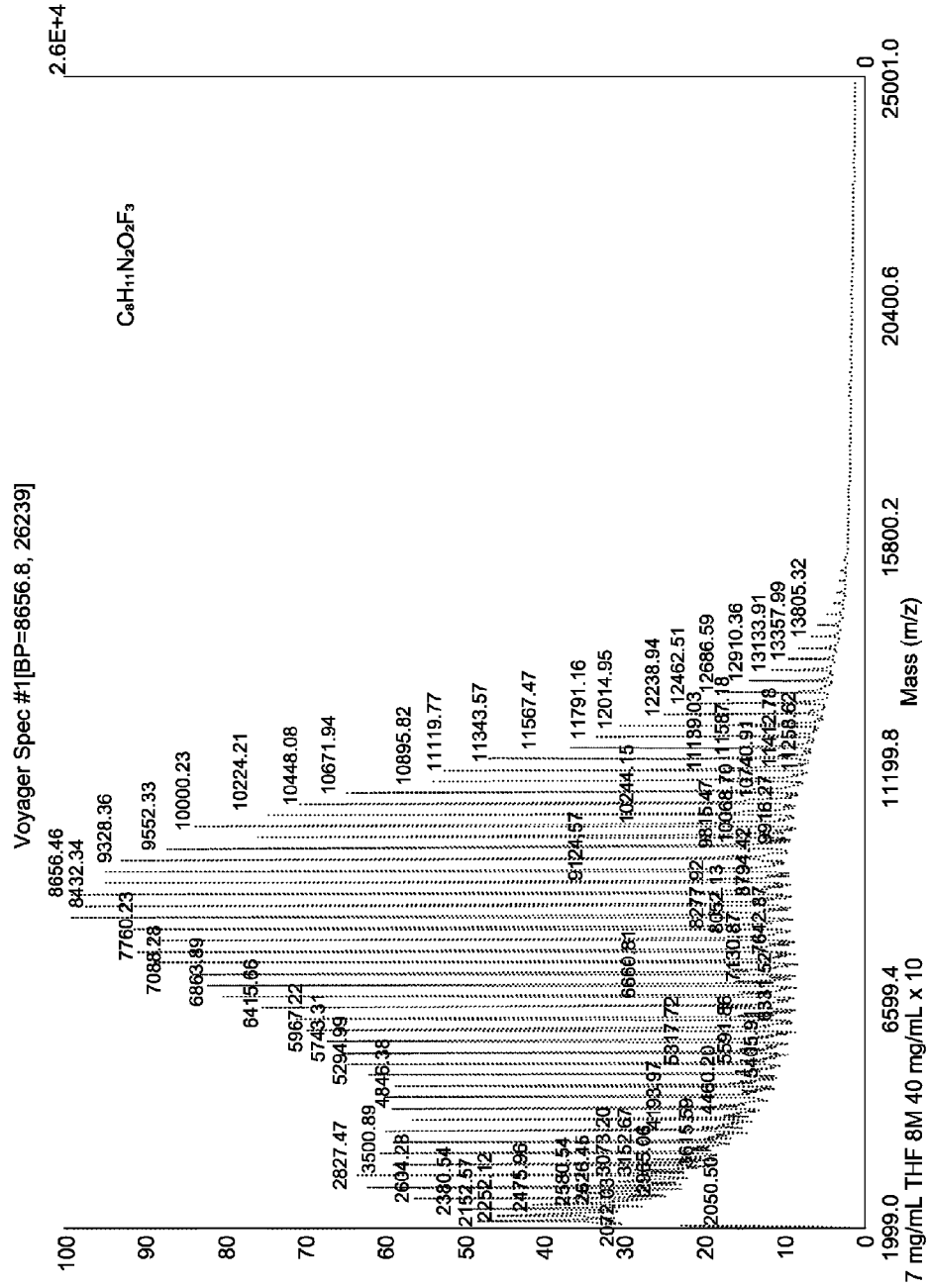

Voyager Spec #1[BP=8656.8, 26239]
$C_8H_{11}N_2O_2F_3$
2.6E+4
0
8656.46
8432.34
9328.36
7760.23
9552.33
10000.23
10224.21
10448.08
10671.94
10895.82
11119.77
11343.57
11567.47
11791.16
12014.95
12238.94
12462.51
12686.59
12910.36
13133.91
13357.99
13805.32
2827.47
3500.89
2604.28
2380.54
2152.57
2252.12
2475.96
2050.50
1999.0
6599.4
11199.8
15800.2
20400.6
25001.0
Mass (m/z)
100
90
80
70
60
50
40
30
20
10
0
7 mg/mL THF 8M 40 mg/mL x 10
L:\...\K30_050212_0001.dat
Acquired: 13:09:00, May 02, 2012

FIG. 7A

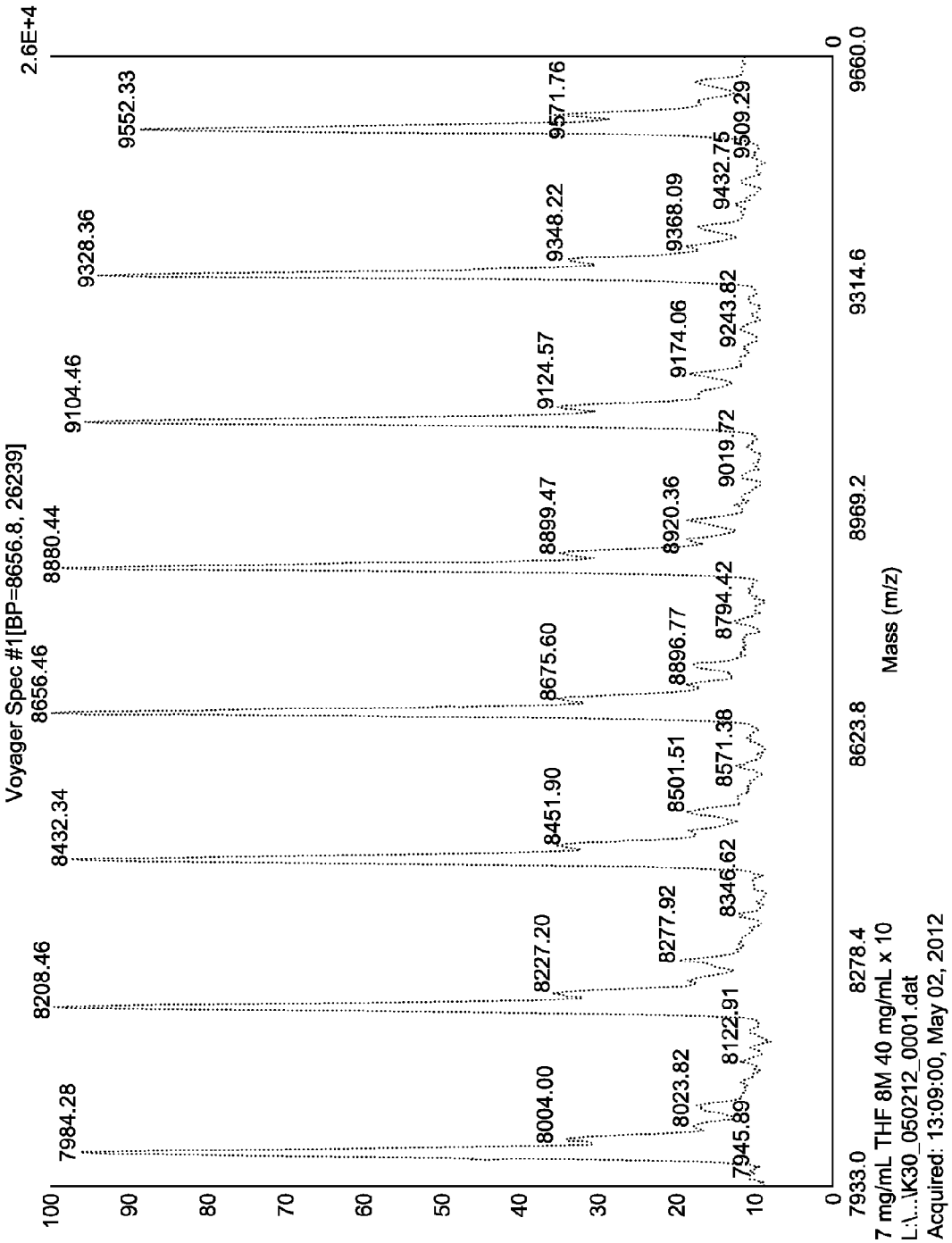

Voyager Spec #1[BP=8656.8, 26239]
2.6E+4
0
100
90
80
70
60
50
40
30
20
10
0
7933.0
8278.4
8623.8
8969.2
9314.6
9660.0
Mass (m/z)
7984.28
8208.46
8432.34
8656.46
8880.44
9104.46
9328.36
9552.33
7945.89
8004.00
8023.82
8122.91
8227.20
8277.92
8346.62
8451.90
8501.51
8571.38
8675.60
8896.77
8794.42
8899.47
8920.36
9019.72
9124.57
9174.06
9243.82
9348.22
9368.09
9432.75
9509.29
9571.76
7 mg/mL THF 8M 40 mg/mL x 10
L:\...\K30_050212_0001.dat
Acquired: 13:09:00, May 02, 2012

FIG. 7B

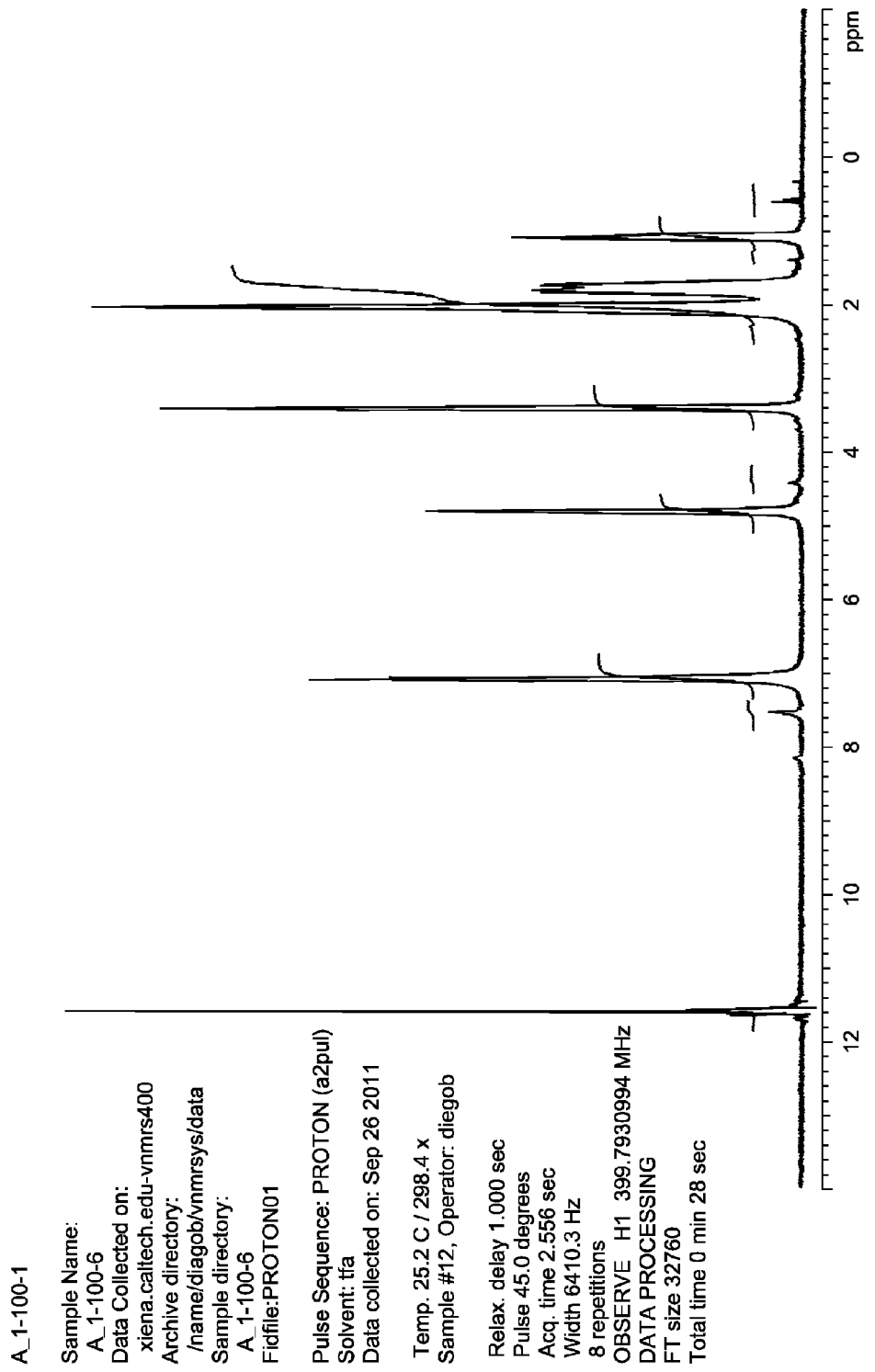

A_1-100-1
Sample Name:
A_1-100-6
Data Collected on:
xiena.caltech.edu-vnmrs400
Archive directory:
/name/diagob/vnmrsys/data
Sample directory:
A_1-100-6
Fidfile:PROTON01
Pulse Sequence: PROTON (a2pul)
Solvent: tfa
Data collected on: Sep 26 2011
Temp. 25.2 C / 298.4 x
Sample #12, Operator: diegob
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 2.556 sec
Width 6410.3 Hz
8 repetitions
OBSERVE H1 399.7930994 MHz
DATA PROCESSING
FT size 32760
Total time 0 min 28 sec
12
10
8
6
4
2
0
ppm

FIG. 8

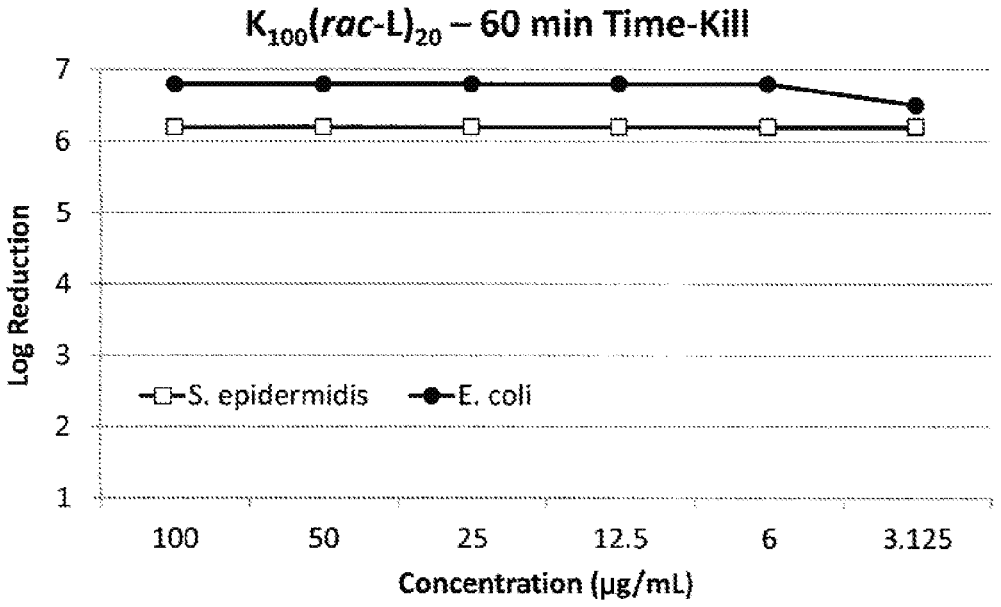

K100(rac-L)20 – 60 min Time-Kill
Log Reduction
7
6
5
4
3
2
1
S. epidermidis
E. coli
100
50
25
12.5
6
3.125
Concentration (µg/mL)

FIG. 9

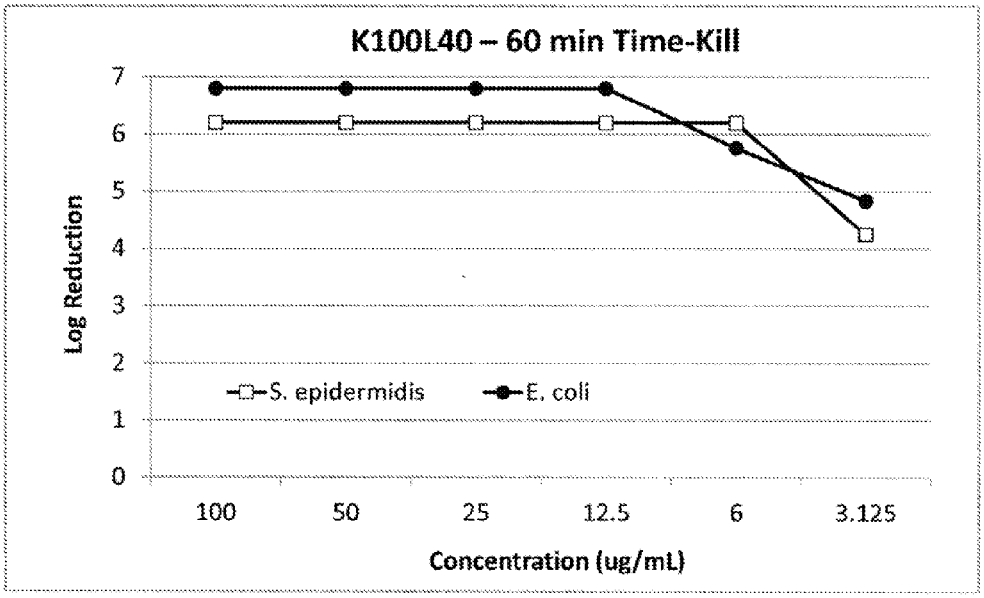

K100L40 – 60 min Time-Kill
Log Reduction
7
6
5
4
3
2
1
0
S. epidermidis
E. coli
100
50
25
12.5
6
3.125
Concentration (ug/mL)

FIG. 10

	10 μg/mL		100 μg/mL	
	5 min	60 min	5 min	60 min
S. aureus	4	≥6	≥6	5
P. aeruginosa	5	≥6	≥6	≥6

	10 μg/mL*		100 μg/mL**	
	5 min	60 min	5 min	60 min
S. aureus	4	≥6	≥6	≥6
P. aeruginosa	4	≥6	≥6	≥6

* With 10 μg/mL poloxamer407
** With 100 μg/mL poloxamer407

FIG. 11

$K_{100}(rac\text{-}L)_{20}$		Log Reduction - 60 min	
		10 µg/mL	100 µg/mL
Gram-positive bacteria	Staphylococcus aureus (MRSA)	5	5
	Staphylococcus epidermidis	≥6	5
	Enterococcus faecium (VRE)	5	≥6
Gram-negative bacteria	Acinetobacter baumannii	≥6	≥6
	Bacteroides fragilis	2	≥6
	Enterobacter cloacae	5	≥6
	Escherichia coli	≥6	≥6
	Klebsiella pneumoniae	≥6	5
	Proteus mirabilis	≥6	≥6
	Serratia marcescens	≥6	≥6
Fungi	Candida albicans	≥5	≥5

FIG. 12

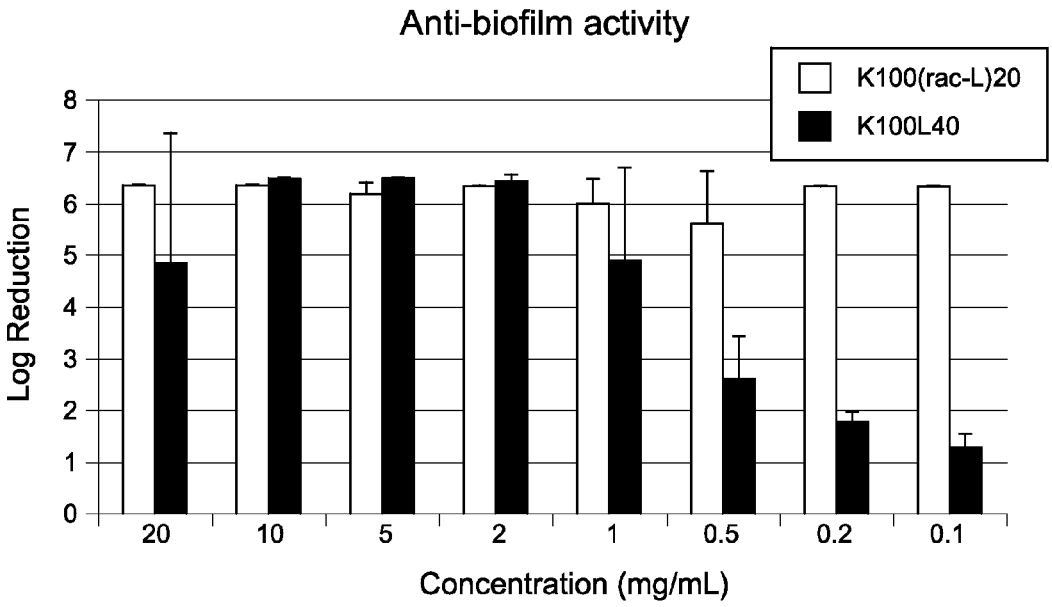
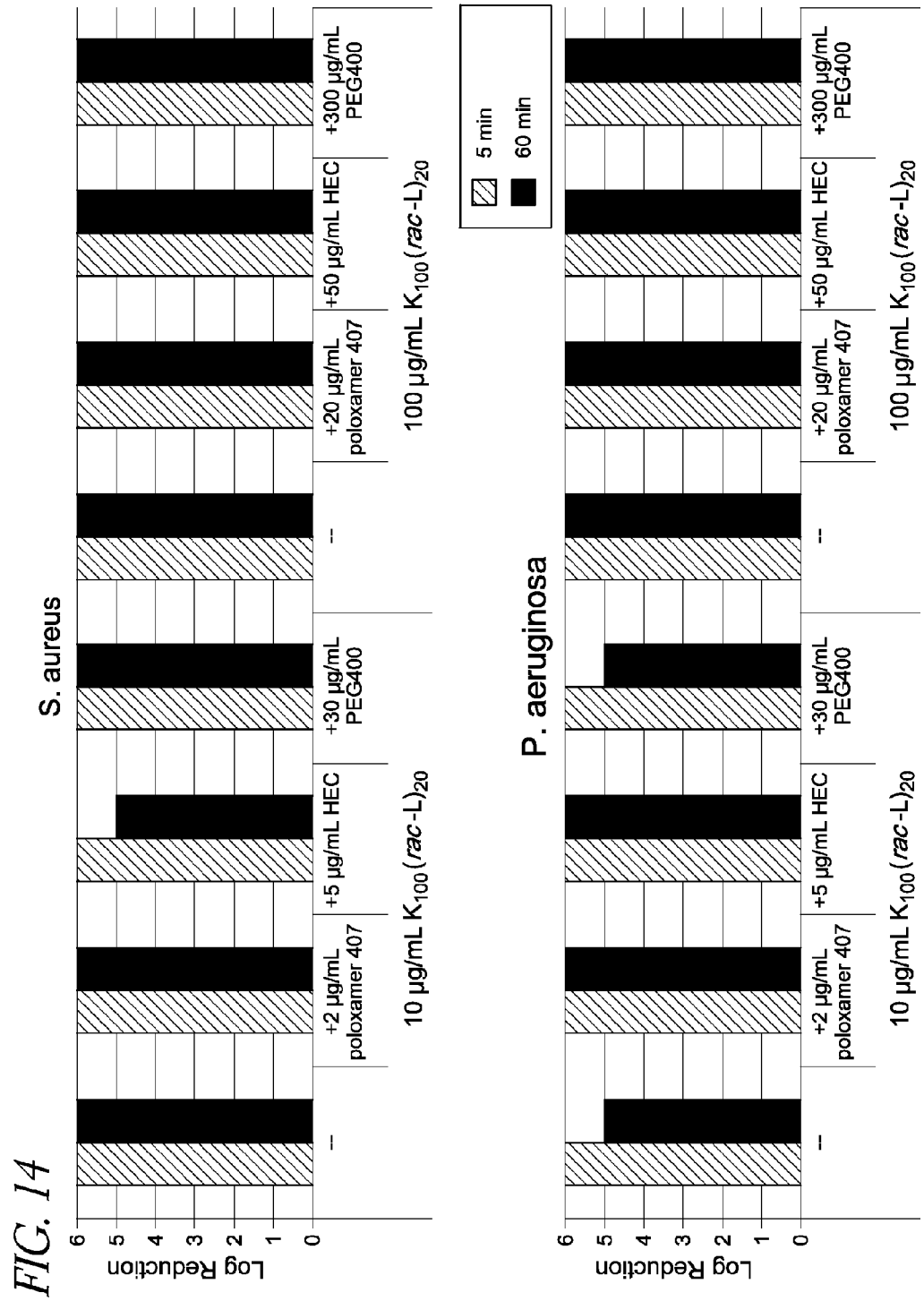

Anti-biofilm activity
K100(rac-L)20
K100L40
Log Reduction
0
1
2
3
4
5
6
7
8
20
10
5
2
1
0.5
0.2
0.1
Concentration (mg/mL)

FIG. 13

FIG. 14


S. aureus
P. aeruginosa
Log Reduction
0
1
2
3
4
5
6
5 min
60 min
--
+2 µg/mL poloxamer 407
+5 µg/mL HEC
+30 µg/mL PEG400
--
+20 µg/mL poloxamer 407
+50 µg/mL HEC
+300 µg/mL PEG400
10 µg/mL K100(rac-L)20
100 µg/mL K100(rac-L)20

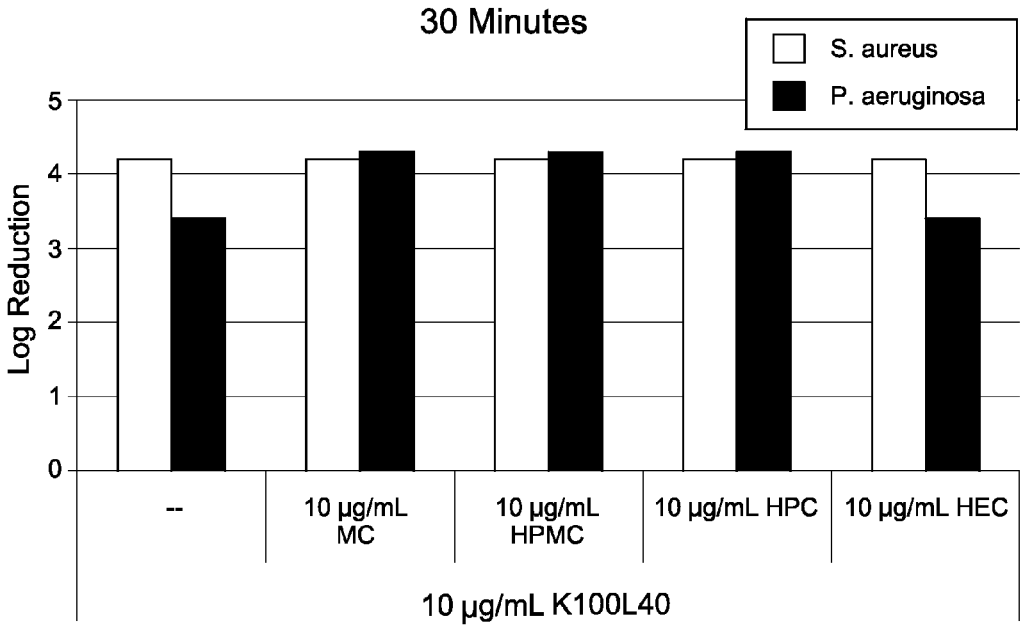

30 Minutes
S. aureus
P. aeruginosa
Log Reduction
5
4
3
2
1
0
--
10 µg/mL MC
10 µg/mL HPMC
10 µg/mL HPC
10 µg/mL HEC
10 µg/mL K100L40

FIG. 15

		S. aureus	S. epidermidis	P. aeruginosa	E. coli
10 µg/mL K100L40	--	1	6	≥6	5
	20 µg/mL HEC	2	≥7	5	5
	200 µg/mL HEC	3	≥6	4	5
100 µg/mL K100L40	--	1	6	≥6	≥6
	200 µg/mL HEC	3	≥7	≥6	≥6
	2000 µg/mL HEC	3	≥7	5	≥6

FIG. 16

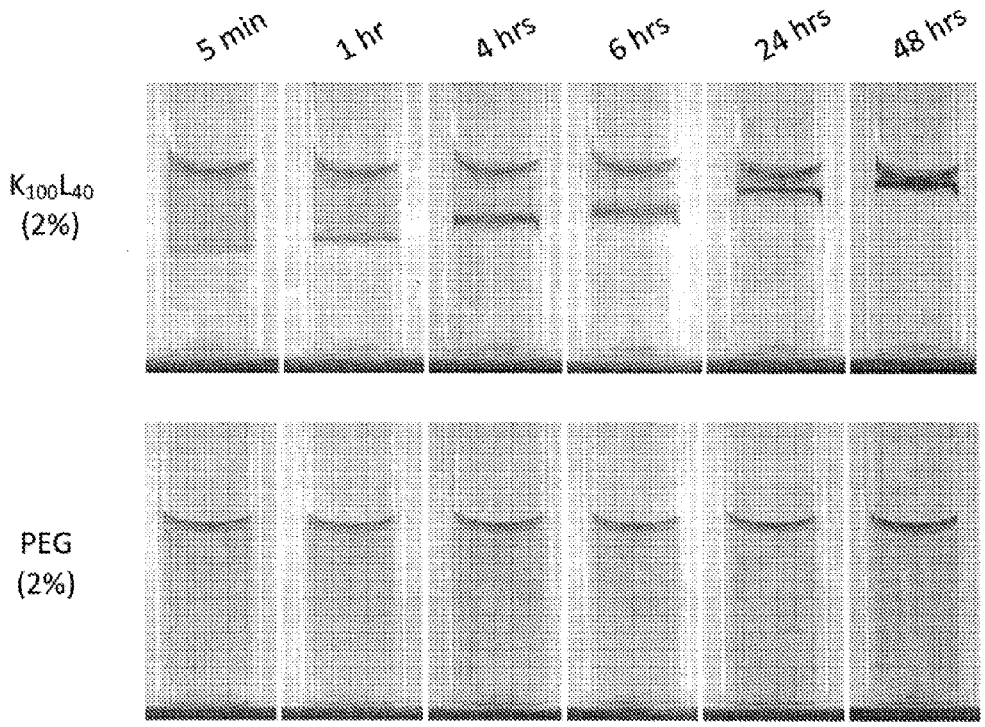


FIG. 17

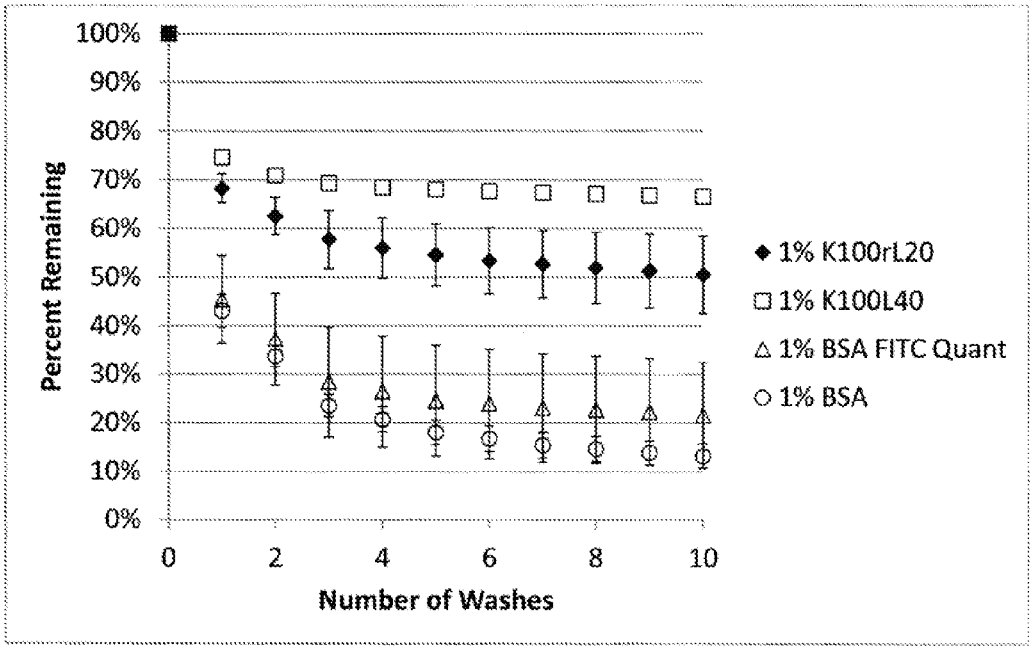

100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent Remaining
0
2
4
6
8
10
Number of Washes
1% K100rL20
1% K100L40
1% BSA FITC Quant
1% BSA

FIG. 18

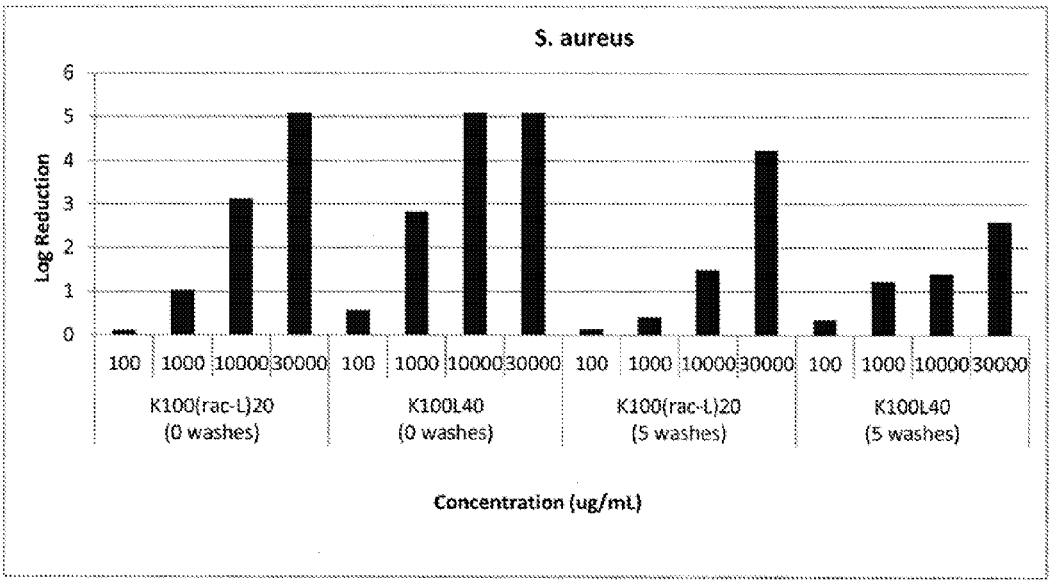

S. aureus
Log Reduction
0
1
2
3
4
5
6
100
1000
10000
30000
K100(rac-L)20
(0 washes)
K100L40
(0 washes)
K100(rac-L)20
(5 washes)
K100L40
(5 washes)
Concentration (ug/mL)

FIG. 19

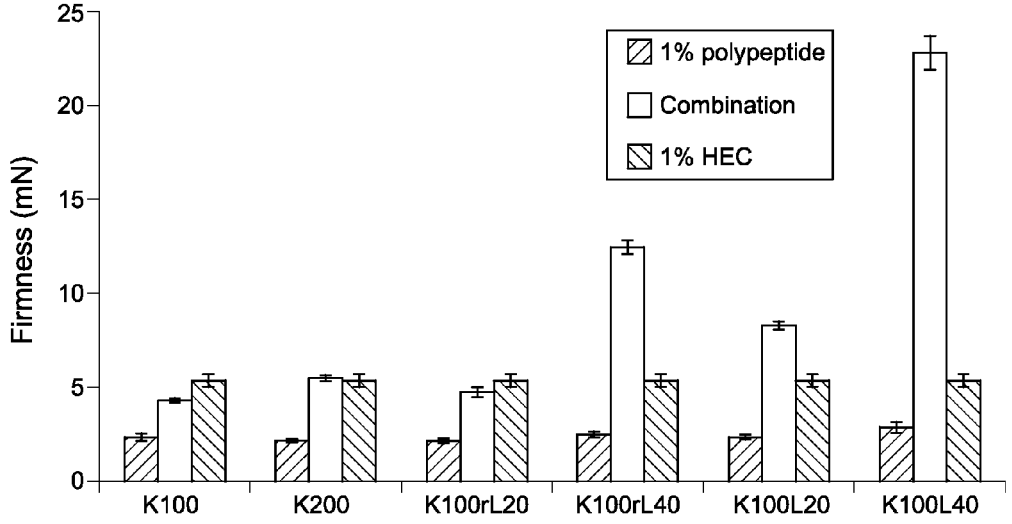


FIG. 20A

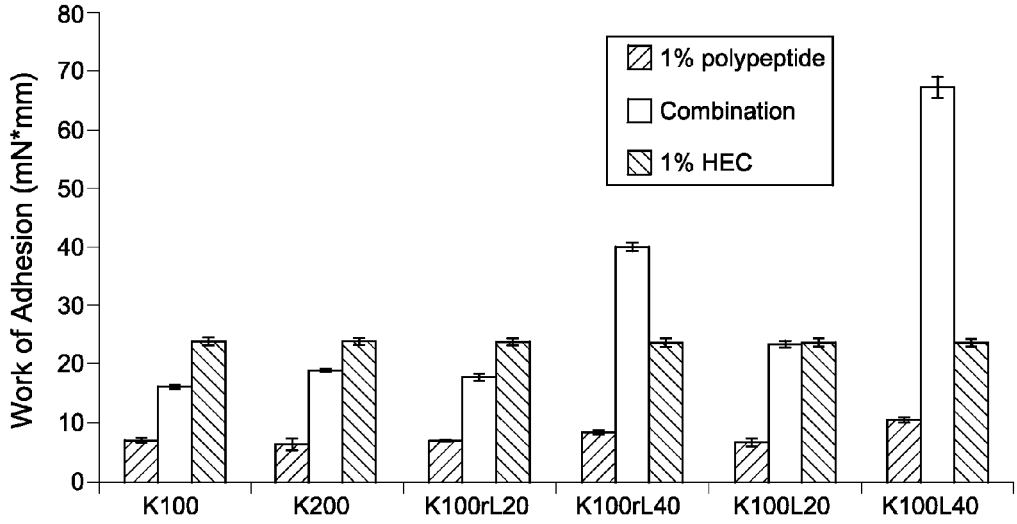


FIG. 20B

Polymer	Firmness[a] (+/- s.d.) (mN)	Firmness (+/- s.d.) w/ HEC[b] (mN)	Firmness Interaction Parameter (mN)[c]	Work of Adhesion[a] (+/- s.d.) (mN•mm)	Work of Adhesion w/HEC[b] (+/- s.d.) (mN•mm)	Work of Adhesion Interaction Parameter (mN•mm)[c]
K_{100}	2.34 (0.20)	4.34 (0.07)	-3.38	6.92 (0.41)	16.21 (0.30)	-14.61
K_{200}	2.17 (0.07)	5.49 (0.16)	-2.06	6.21 (1.17)	18.92 (0.21)	-11.20
$K_{100}(rac\text{-}L)_{20}$	2.18 (0.11)	4.76 (0.25)	-2.80	6.99 (0.08)	17.93 (0.56)	-12.96
$K_{100}(rac\text{-}L)_{40}$	2.51 (0.13)	12.47 (0.34)	4.58	8.64 (0.18)	40.19 (0.63)	7.64
$K_{100}L_{20}$	2.37 (0.14)	8.29 (0.19)	0.55	6.80 (0.70)	23.64 (0.48)	-7.07
$K_{100}L_{40}$	2.94 (0.25)	22.77 (0.90)	14.45	22.60 (0.59)	79.43 (2.33)	32.93
HEC	5.38 (0.32)	-	-	23.90 (0.62)	-	-

FIG. 21

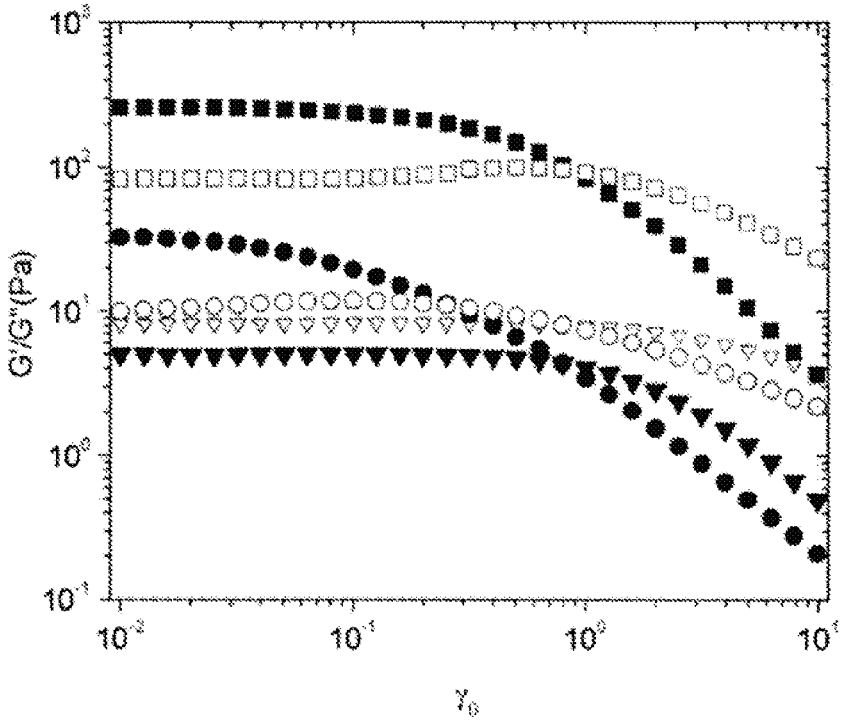


FIG. 22A

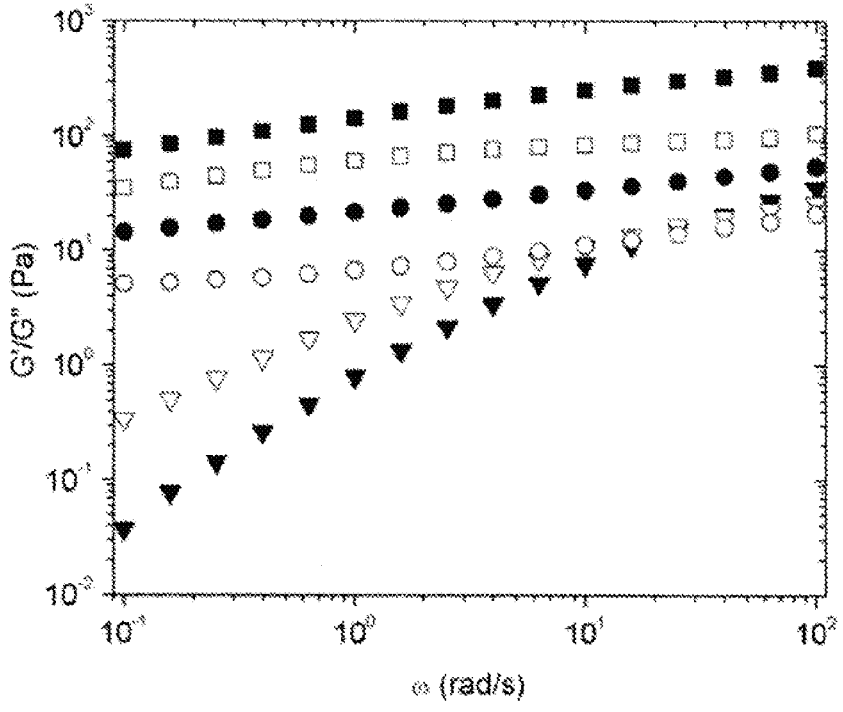


FIG. 22B

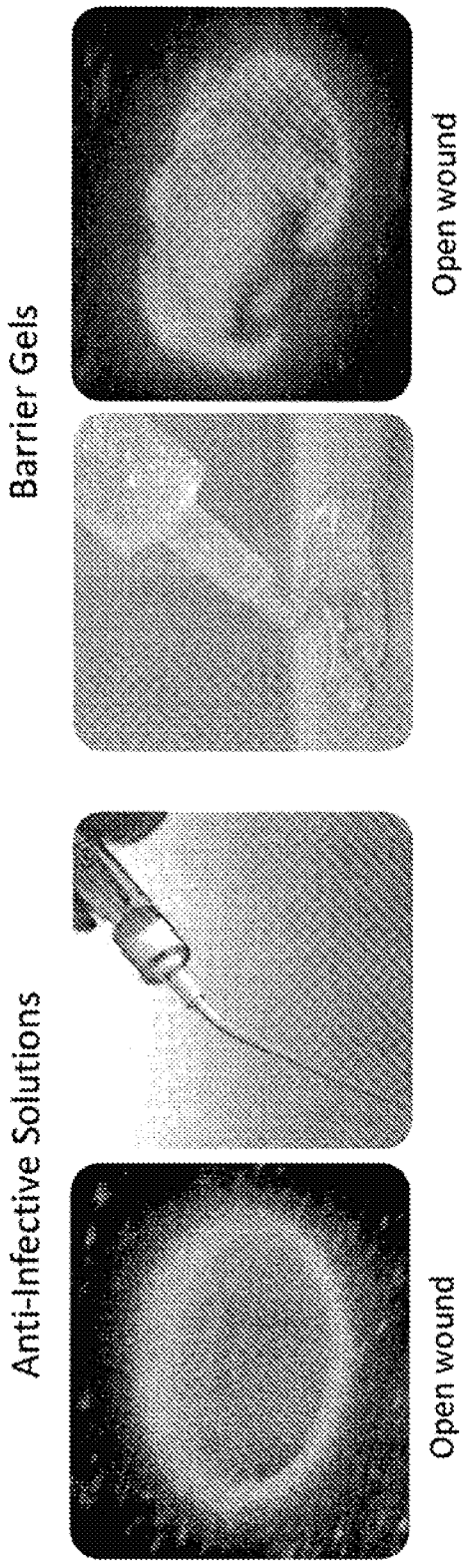

Anti-Infective Solutions
Barrier Gels
Open wound
Open wound

FIG. 23

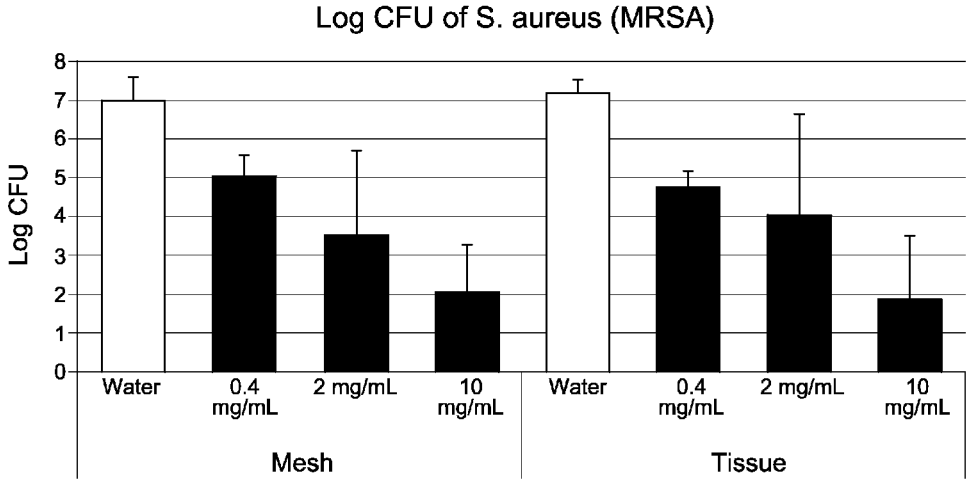

Log CFU of S. aureus (MRSA)
Log CFU
8
7
6
5
4
3
2
1
0
Water
0.4 mg/mL
2 mg/mL
10 mg/mL
Water
0.4 mg/mL
2 mg/mL
10 mg/mL
Mesh
Tissue

FIG. 24

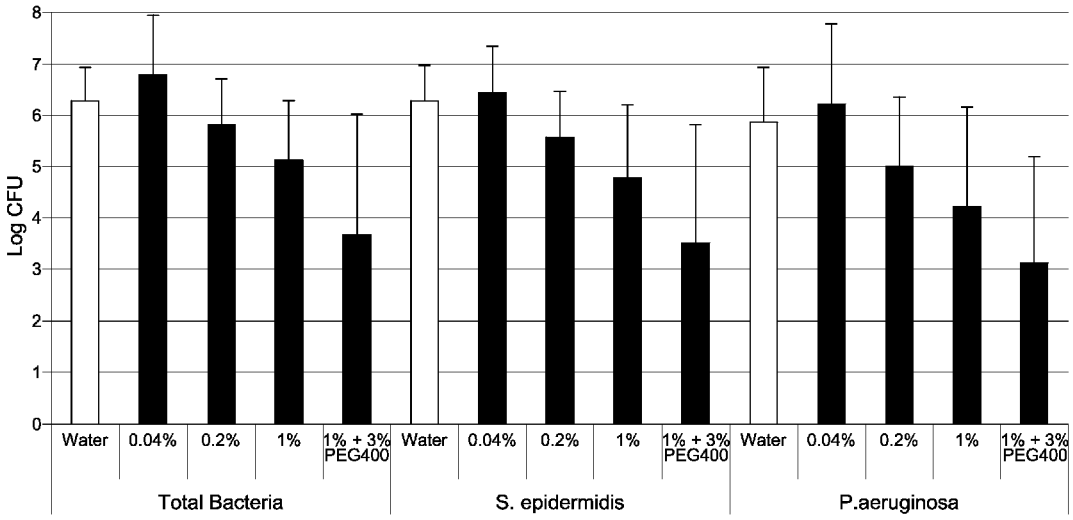

Log CFU
0
1
2
3
4
5
6
7
8
Water
0.04%
0.2%
1%
1% + 3% PEG400
Water
0.04%
0.2%
1%
1% + 3% PEG400
Water
0.04%
0.2%
1%
1% + 3% PEG400
Total Bacteria
S. epidermidis
P.aeruginosa

FIG. 25

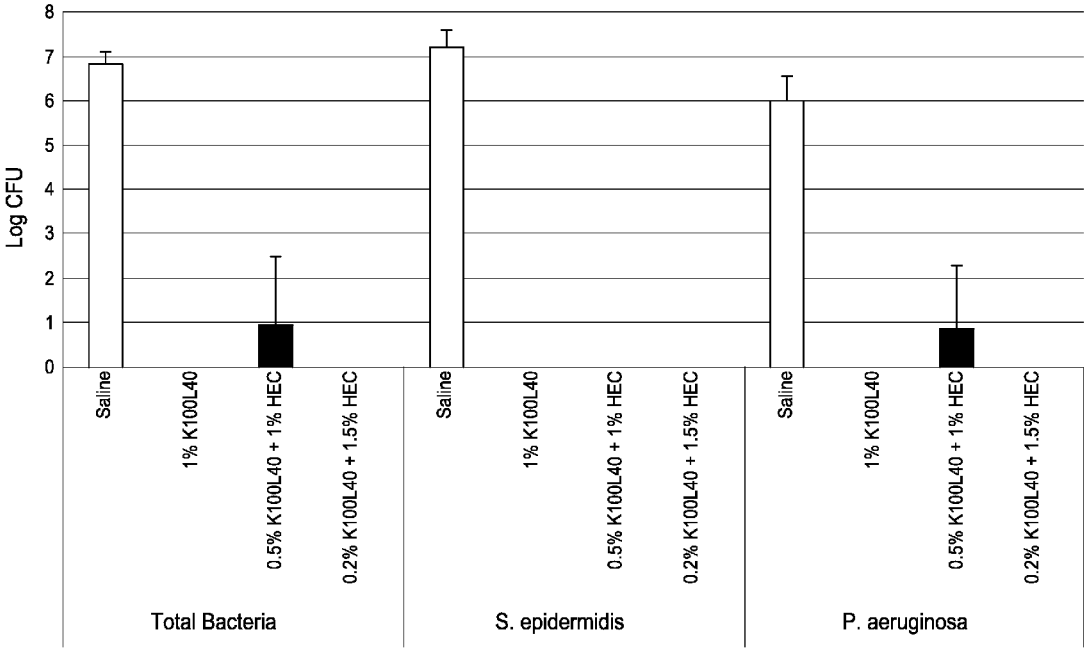

Log CFU
8
7
6
5
4
3
2
1
0
Saline
1% K100L40
0.5% K100L40 + 1% HEC
0.2% K100L40 + 1.5% HEC
Saline
1% K100L40
0.5% K100L40 + 1% HEC
0.2% K100L40 + 1.5% HEC
Saline
1% K100L40
0.5% K100L40 + 1% HEC
0.2% K100L40 + 1.5% HEC
Total Bacteria
S. epidermidis
P. aeruginosa

FIG. 26

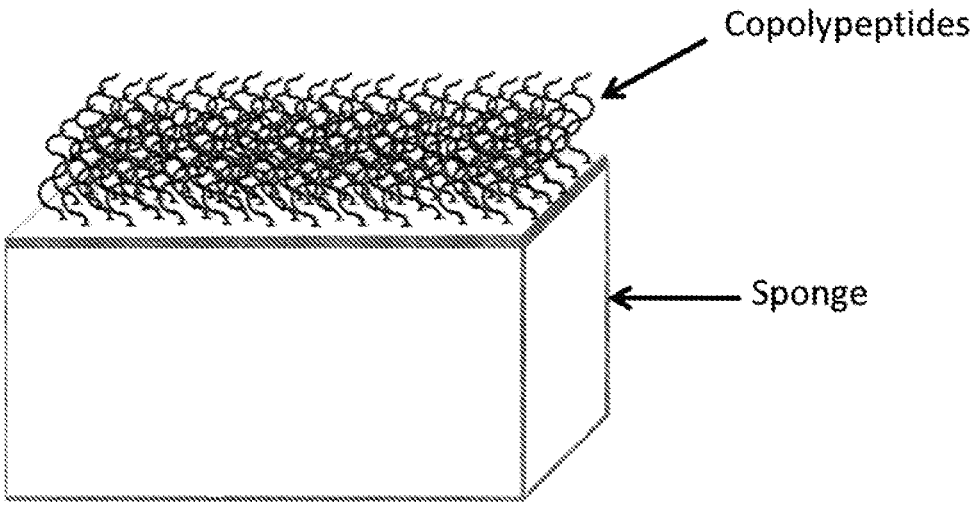


FIG. 27

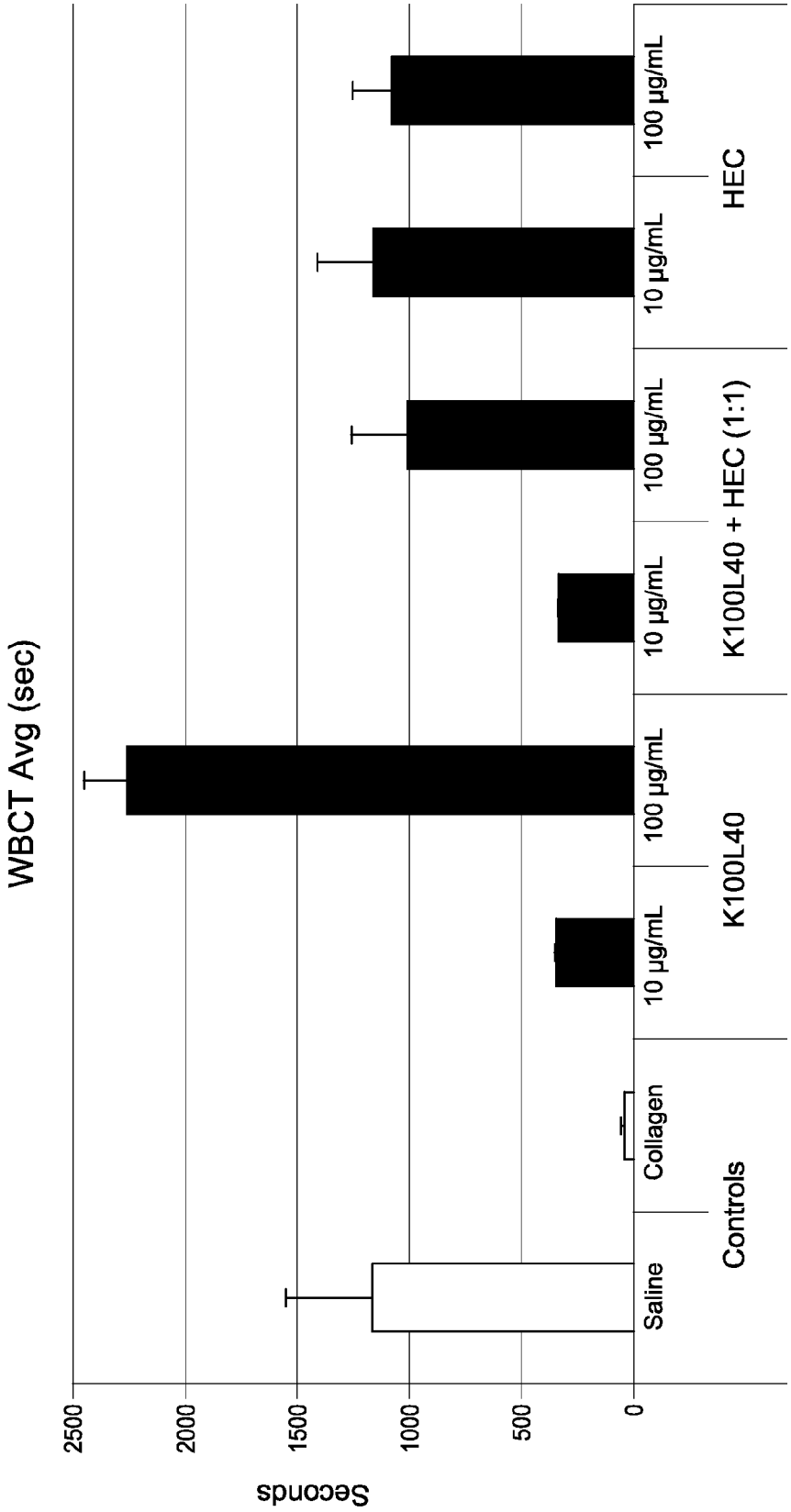

WBCT Avg (sec)
Seconds
2500
2000
1500
1000
500
0
Saline
Collagen
10 µg/mL
100 µg/mL
10 µg/mL
100 µg/mL
10 µg/mL
100 µg/mL
Controls
K100L40
K100L40 + HEC (1:1)
HEC

FIG. 28

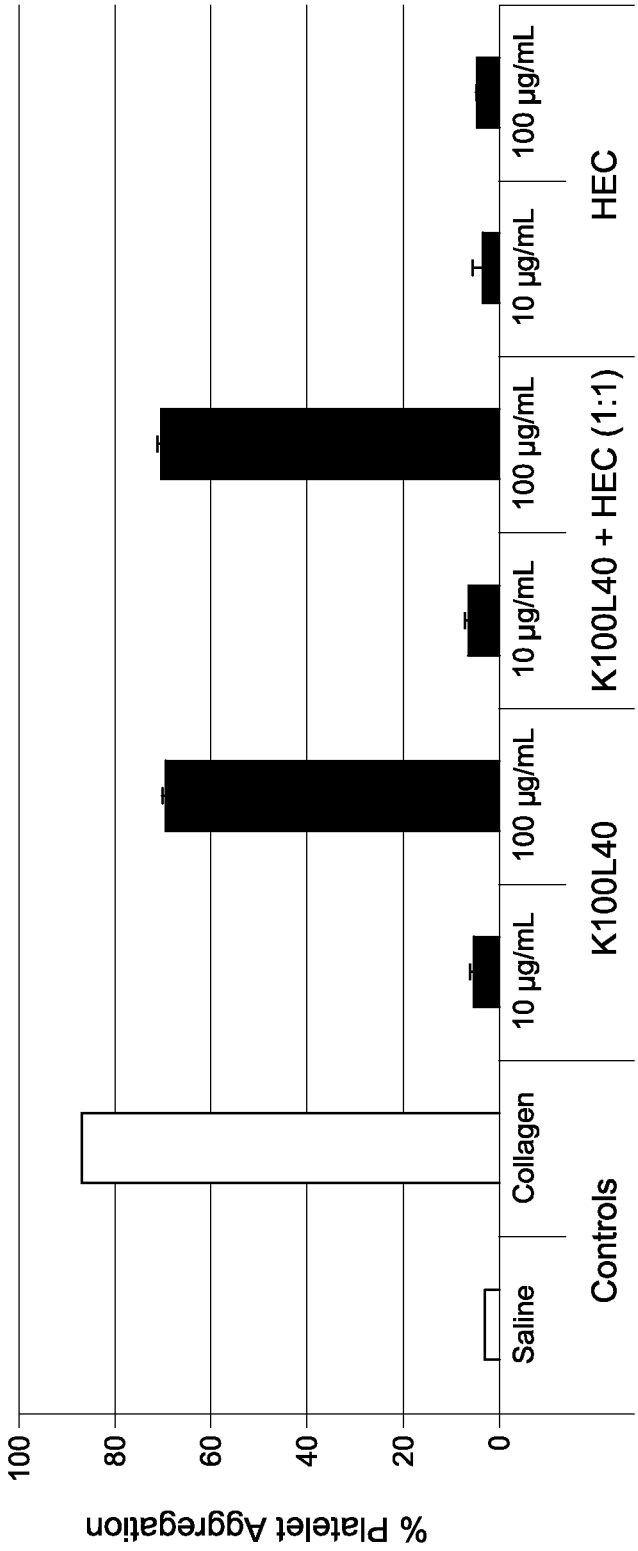

Platelet Aggregation (%)
% Platelet Aggregation
100
80
60
40
20
0
Saline
Collagen
10 µg/mL
100 µg/mL
10 µg/mL
100 µg/mL
10 µg/mL
100 µg/mL
Controls
K100L40
K100L40 + HEC (1:1)
HEC

FIG. 29

COMPOSITIONS AND USES OF ANTIMICROBIAL MATERIALS WITH TISSUE-COMPATIBLE PROPERTIES

INCORPORATION BY REFERENCE TO ANY PRIORITY APPLICATIONS

This application is the U.S. National Phase of International Application PCT/US2013/032535, filed Mar. 15, 2013, which claims the benefit of priority to U.S. Provisional Application No. 61/615,150, filed on Mar. 23, 2012, U.S. Provisional Application No. 61/625,757, filed on Apr. 18, 2012, U.S. Provisional Application No. 61/625,760, filed on Apr. 18, 2012, and U.S. Provisional Application No. 61/716,242, filed on Oct. 19, 2012, the disclosures of each of which are hereby incorporated by reference in their entireties.

BACKGROUND OF THE INVENTION

When barriers are broken, infections occur. Surgery, trauma and burns, medical instrumentation (e.g., catheterization, ventilation), chronic wounds (e.g., diabetic foot ulcers) and a variety of diseases disrupt our natural barriers of defense. In nearly all cases, these "wounds" become contaminated with microbes. Then, the terrain of wounds provides an excellent environment for microbial growth. The battle begins, and it's trench warfare. Damaged tissues ("cracks and crevices"), altered blood flow and exudate production, changes in local temperature, pH and tissue oxygenation, as well as the lack of commensal bacteria, can all contribute. Also, bleeding and vascular leakage may provide fluids and nutrients that ultimately support microbial growth. Bacterial or fungal colonization, and/or overt infection may occur. Microbial biofilms can help microbes to create their own local environments. Various surgical, trauma and medical settings all involve disruption of our natural barriers of defense and deserve special attention because the outcomes can range from rapid cure to lethal sepsis.

Natural barriers are typically referred to by their anatomical sites such as skin, pulmonary epithelium, gastrointestinal mucosa, etc. These names may imply a level of simplicity that is unwarranted. These barriers are often both passive and active. They can involve a variety of cells, secreted glycoproteins, matrix components and fluids that act in concert to provide effective defense against microbial invasion. In some sites, resident microbes contribute to the barrier action against other potential invaders. Under most circumstances, these physical and functional barriers are highly effective. However, they can be broken rather easily by mechanical or chemical insults. In addition, certain systemic diseases can weaken our natural barriers and increase the risk of breakdown, as occurs in diabetic foot ulcers or cystic fibrosis. Finally, a first infection can weaken host defenses against a second infection, as occurs in influenza followed by bacterial pneumonia or *trichomonas vaginalis* followed by certain sexually transmitted diseases (e.g., HIV).

Broken barriers of defense leave the host susceptible to infection by a wide variety of microbes, ranging from typically benign commensal organisms to aggressive pathogens. Commonly, we are our own source of the microbes that contaminate our wounds. The human body hosts a very large number of bacteria, predominately on skin, in mouths and within lower GI tracts. It has been estimated that there are more bacterial cells (10^{14}) than mammalian cells (10^{13}) within the space of one human body. Despite this close relationship with microbes, most of our tissues (including blood, subcutaneous tissue, muscle, brain) remain sterile until the disruption of the natural barriers. Other people and environmental sources of microbes are also important, especially in healthcare settings. Once a barrier is broken, microbial contamination, critical level colonization, biofilm formation and/or overt infection may occur. Polymicrobial colonization and/or infection is common in certain settings (e.g., diabetic foot ulcers, complex intra-abdominal infections), and may involve aerobes, anaerobes or both.

Prior approaches to the prevention and treatment of these infections have demonstrated substantial weaknesses. Both lack of effectiveness and tissue toxicity have been challenges. Antimicrobials often fail to get to the right tissue spaces and/or fail to remain active for sufficient time to prevent or treat infection. Complex surfaces like those of the abdominal cavity or large burns are particularly difficult to cover effectively. Finally, safe application of sufficient antimicrobial materials into certain tissue spaces (such as through laparoscopic or arthroscopic equipment) can be challenging. Antimicrobials that are readily applied by these methods tend to be solution-based materials with limited ability to bind tissues and remain active over time.

Biofilms present a particular challenge. Increasing evidence points to the resistance of bacterial biofilms to a variety of antimicrobial approaches and to their role in adverse patient outcomes. These microbial communities resist traditional antiseptics and antibiotics through several mechanisms, including, but not limited to, their own production of extracellular polymeric substances, which are often negatively charged (anionic). Penetration of these materials by traditional antimicrobials is often limited. For example, in acute wounds (e.g. surgery and trauma), devitalized tissue and foreign bodies (e.g., prosthetic implants) may support biofilm formation and thereby increase the probability of overt infection. In chronic wounds (e.g. diabetic foot ulcers), biofilms may persist and lead to delayed wound healing. Medical instruments like ventilators and catheters can be a site of biofilm formation and provide a source of infection.

Antimicrobial treatment of early infections may alter the course of the infection, resulting in more resistant and more dangerous infections. Common antimicrobial strategies focus on the use of selective antibiotics (e.g., penicillin for gram-positive organisms) in order to avoid the development of bacteria that are resistant to broad-spectrum antibiotics. Inadvertently, this important strategy can have negative outcomes on an individual patient, where targeted antibiotics result in the emergence of an aggressive, different microorganism (e.g., *Pseudomonas*). In this way, treated wounds can become the site for a "parade of pathogens", where an early, dominant microbial species (e.g., *Staph aureus*) is replaced by a second (e.g., MRSA, methicillin-resistant *Staph aureus*) and, perhaps, even a third and fourth microbial species (e.g., a multi-drug resistant gram negative species).

The large numbers of adverse patient outcomes in today's advanced healthcare settings underscore the inadequacies of prior art in the prevention and treatment of these infections. Several key weaknesses include:

1. Low antimicrobial activity in tissue settings and on biofilms;
2. Inadequate distribution to the relevant tissue space;
3. Limited, if any, barrier activity;
4. Narrow breadth of antimicrobial activity enables the "parade of pathogens";

5. Inadequate treatment fosters more antimicrobial resistance; and/or

6. Tissue toxicity.

Infections of wounds and other broken-barrier settings are common and costly. In the US alone, approximately 12 million traumatic injuries are treated in emergency departments each year. In addition, there are more than 50 million surgeries (inpatient and outpatient). The US Department of Health and Human Services indicates that there are more than 1.7 million healthcare-associated infections annually, resulting in approximately 100,000 deaths and $30 billion in healthcare costs per year. Many of these healthcare-associated infections start with broken barriers. Examples include surgical site infections (SSIs), catheter-associated urinary tract infections and ventilator-associated pneumonia. The chronic wounds associated with pressure ulcers (bed sores) and diabetic foot ulcers present their own unique challenges.

In addition to infection, several other wound-associated outcomes remain major challenges. These include blood loss, tissue adhesions/scarring, and poor wound healing. And, in some cases, known antimicrobial treatments make these problems worse. Certain antimicrobial wound treatments (including antibiotic washes) can result in excessive tissue responses (e.g., tissue adhesions or scarring). Certain antiseptic/antimicrobial materials may alter wound healing, resulting in insufficient tissue responses (e.g., poor wound healing, poor wound strength).

Effective hemostasis in wounds also remains a substantial problem. Hemostatic materials have been described and are utilized in a variety of settings, including in trauma and in surgery. While effective in some situations, these materials do not provide ideal solutions to the challenges. First, there are times when the hemostasis is insufficient and too much bleeding occurs, potentially with lethal consequences. In some of these cases initial hemostasis occurs, however, subsequent re-bleeding occurs. This may be due to fibrinolytic activity. In addition to the problems resulting from blood loss, extravasated blood components in the tissues may contribute to additional adverse outcomes including infection and the fibrotic responses seen with post-surgical tissue adhesions. Second, in some cases, hemostatic materials cause problems by entering the blood stream and causing clotting (thrombosis) within blood vessels, potentially, with lethal outcomes. Third, in some cases, wound treatment materials (including hemostatic materials) can serve as a site for subsequent infection or can result in abnormal tissue responses such as adhesion formation and/or tissue scarring, resulting in adverse medical outcomes. Improved approaches to hemostasis are needed.

SUMMARY OF THE INVENTION

In accordance with embodiments of the invention, a mixture of an antimicrobial cationic polypeptide and a second pharmaceutically-acceptable polymer is used in the treatment and prevention of infections that occur when our natural barriers of defense are broken. These novel compositions can provide two functions: direct antimicrobial activity and barrier activity. Embodiments of the invention addresses one or more weaknesses of previous antimicrobials described above. Notably, it has been recognized that the effectiveness of most of the previous antiseptics and antibiotics were based on determinations of antimicrobial activity in solution with the microbes in suspension (MIC assays), producing results that are not necessarily indicative of effectiveness in tissues, and thus could lead away from determining effectiveness in tissues. By contrast, the inventors focused on the design and selection of agents for broad antimicrobial activity, especially at tissue surfaces and in the terrain ("cracks and crevices") of wounds. This includes the formation of a barrier containing cationic (positively charged) elements that can inhibit the movement of certain substances or cells that display anionic elements (e.g., microbes). In one embodiment, the synthetic cationic polypeptides of these compositions contain at least one cationic segment and at least one hydrophobic segment, are comprised substantially of natural amino acids, and are broadly antimicrobial (i.e., against gram positive and gram negative bacteria). They can also be designed to self-assemble, based in part on the interaction of their hydrophobic segments. Further, synthetic polypeptides are formulated with a second pharmaceutically-acceptable polymer to provide a composition that is directly antimicrobial and that effectively coats tissues. These mixtures may also display hemostatic properties. In some embodiments, the second pharmaceutically acceptable polymer is not a polyethylene glycol (PEG).

Embodiments of the invention may be used alone or in combination with other materials that provide similar or complementary activities.

An embodiment provides aqueous composition for the prevention, inhibition, or treatment of infection comprising: a mixture comprising one or more synthetic, cationic polypeptide(s) with antimicrobial activity; and a second pharmaceutically-acceptable polymer that is not a synthetic, cationic polypeptide(s) with antimicrobial activity; wherein the amounts of the one or more synthetic, cationic polypeptide(s) and the second pharmaceutically-acceptable polymer are each at least about 100 μg/mL based on the total volume of the aqueous composition; wherein the amount of the second pharmaceutically-acceptable polymer is at least about 10% by weight, based on the weight of the one or more synthetic, cationic polypeptide(s); and wherein the synthetic, cationic polypeptide(s) and the second pharmaceutically-acceptable polymer are mutually miscible in water.

The synthetic, cationic polypeptide(s) with antimicrobial activity and the second pharmaceutically-acceptable polymer are considered mutually miscible if at least about 90% of the polymeric components remain mutually soluble 24 hours after mixing and maintaining at room temperature in water at a concentration of each polymer of 1 mg/mL, upon visible examination.

In another embodiment, one or more of the synthetic cationic polypeptide(s) in the aqueous composition comprises a segment having a chain length of at least 40 amino acid residues.

In another embodiment, the synthetic cationic polypeptide(s) in the aqueous composition comprises substantially all natural amino acid subunits.

In another embodiment, the synthetic cationic polypeptide(s) in the aqueous composition is characterized by at least one segment containing at least five consecutive cationic amino acid residues and at least one segment containing at least five consecutive hydrophobic amino acid residues.

In another embodiment, the second pharmaceutically-acceptable polymer in the aqueous composition is selected from the group consisting of cellulose, alginate, collagen, polymeric surfactant, polyethylene glycol, polyvinyl alcohol, polyurethane, polyvinyl pyrolidinone (PVP), fibrin(ogen), blood proteins and tissue proteins.

In another embodiment, the antimicrobial activity of the aqueous composition is greater than 3 logs killing of *Staphylococcus epidermidis* and *Escherichia coli* in standard 60

minute time-kill assays at a synthetic cationic polypeptide(s) concentration of 100 μg/mL or less.

In another embodiment, the aqueous composition is further characterized by the ability to disrupt or inhibit a biofilm in vitro at a total polymer concentration of 40 mg/ml or less.

In another embodiment, the aqueous composition is further characterized by a barrier activity, as measured by a decrease in the diffusion rate of an anionic dye of more than 2 logs at a total polymer concentration of 40 mg/mL or less.

In another embodiment, the aqueous composition is further characterized by a storage modulus of at least 50 Pa at a total polymer concentration of less than 40 mg/mL.

In another embodiment, the aqueous composition is further characterized by a storage modulus of at least 50 Pa at a total polymer concentration of less than 40 mg/mL and an ability to pass through a 20 g needle using less than 60 N pressure.

In another embodiment, the aqueous composition is further characterized by an ability to pass through a 20 g needle and recover a minimum of 70% of its strength as measured by storage modulus within 10 minutes.

In another embodiment, the aqueous composition is in the form of a solution, a gel, a cream, a foam, or a dressing.

In another embodiment, the aqueous composition is further characterized as being in combination with, or binding to, a dressing material, including but not limited to a gauze or sponge.

In another embodiment, the aqueous composition has pro-coagulant activity, pro-hemostatic activity, or both.

In another embodiment, the aqueous composition further comprises an active pharmaceutical ingredient (API) selected from the group consisting of steroid, pro-inflammatory agent, anti-inflammatory agent, anti-acne agent, preservatives hemostatic agent, angiogenic agent, wound healing agent, anti-cancer agent and other antimicrobial agent.

Another embodiment provides a use of any one of the aqueous compositions described herein for any one or more selected from the group consisting of prevention of infections, treatment of infections, treatment for topical anti-infection, treatment for microbial decolonization, wound treatment, surgical site treatment, trauma treatment, burn treatment, treatment of diabetic foot ulcers, eye treatment, treatment of vaginal infections, treatment of urinary tract infections, hand sanitization, for coating prosthetic devices and/or implants, food preservation and solution preservation.

Another embodiment provides a method for the prevention and/or treatment of infections comprising: contacting a tissue of a patient subject with any of the aqueous compositions described herein.

In another embodiment, the method further comprises applying negative-pressure to a wound.

In another embodiment, the method further comprises treating the patient systemically with other antibiotics and/or locally with another antimicrobial, and/or at least one selected from the group consisting of an antibiotic, an anti-biofilm agent, a surfactant, and a combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts examples of synthetic cationic polypeptides, $K_x(\text{rac-L})_y$ and K_xL_y. These polypeptides contain a cationic segment of lysine amino acids and a hydrophobic segment of leucine amino acids. The cationic segments provide for multimeric interaction with anionic substances, including bacterial surfaces. The hydrophobic segments associate in aqueous media, leading to the formation of various structures, such as multimers in solution, micelles, sheets, and fibrils. These hydrophobic interactions are important in barrier formation.

FIG. **2** is a table (Table 1) that shows examples of synthetic cationic polypeptides synthesized using either (A) an amine or (B) an organometallic $Co(PMe_3)_4$ initiator.

FIG. **3** is a gel permeation chromatogram of a chain extension experiment.

FIG. **4** is a gel permeation chromatogram of a representative deprotected diblock copolypeptide.

FIG. **5** is an attenuated total reflectance fourier-transform infrared (ATR-IR) interferogram of monomers.

FIG. **6** is an attenuated total reflectance fourier-transform infrared (ATR-IR) interferogram of crude reaction mixture and complete polymerization.

FIG. **7** shows a matrix-assisted laser desorption ionization (MALDI) mass spectrum of a representative peptide.

FIG. **8** shows a liquid proton nuclear magnetic resonance (H^1-NMR) spectrum of a representative deprotected copolypeptide in d-TFA.

FIG. **9** shows an in vitro antimicrobial time-kill assay (60 min) against *S. epidermidis* (RMA 18291), and *E. coli* (ATCC 25922) using $K_{100}(\text{rac-L})_{20}$.

FIG. **10** shows an in vitro antimicrobial time-kill (60 min) against *S. epidermidis* (RMA 18291), and *E. coli* (ATCC 25922) using $K_{100}L_{40}$.

FIG. **11** is a table (Table 2) that shows in vitro antimicrobial time-kills of $K_{100}(\text{rac-L})_{20}$ and 1:1 $K_{100}(\text{rac-L})_{20}$: Poloxamer 407 solutions at concentrations of 10 and 100 μg/mL against *S. aureus* (29213), and *P. aeruginosa* (27853) after 5 and 60 min.

FIG. **12** is a table (Table 3) that shows an in vitro antimicrobial time-kill of $K_{100}(\text{rac-L})_{20}$ solutions at concentrations of 10 and 100 μg/mL against a variety of gram positive and gram negative bacteria and fungi after 60 min contact time.

FIG. **13** shows that $K_{100}(\text{rac-L})_{20}$ and $K_{100}L_{40}$ at concentrations of 0.1-20 mg/mL are effective against *P. aeruginosa* biofilms in vitro (24 hour contact time).

FIG. **14** shows an in vitro antimicrobial time-kill assay against *S. aureus* (29213), and *P. aeruginosa* (27853) after 5 and 60 min, using $K_{100}(\text{rac-L})_{20}$ at concentrations of 10 and 100 μg/mL with other ratios of excipients: 5:1 $K_{100}(\text{rac-L})_{20}$:Poloxamer 407, 2:1 $K_{100}(\text{rac-L})_{20}$:HEC, and 1:3 $K_{100}(\text{rac-L})_{20}$:Peg 400.

FIG. **15** shows an in vitro antimicrobial time-kill assay against *S. aureus* (29213) after 30 min, using $K_{100}L_{40}$ (10 μg/mL) alone and in combination with cellulose ethers (methyl cellulose (MC), hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), and hydroxyethyl cellulose (HEC)) at a 1:1 ratio.

FIG. **16** is a table (Table 4) that shows an in vitro antimicrobial time kill assay against *S. aureus* (29213), *S. epidermidis* (RMA 18291), *P. aeruginosa* (27853), and *E. coli* (25922) after 60 min. contact time. $K_{100}L_{40}$ at concentrations of 10 and 100 μg/mL with other ratios of hydroxyethyl cellulose: 1:2 $K_{100}(\text{rac-L})_{20}$:HEC and 1:20 $K_{100}(\text{rac-L})_{20}$ were tested.

FIG. **17** shows a barrier assay where a 2% synthetic cationic polypeptide preparation ($K_{100}L_{40}$) was shown to be highly effective in blocking the diffusion of a colored, anionic dye over a 48 hour period. By contrast, the dye diffused readily (within 5 minutes) through 2% polyethylene glycol (10,000) preparation.

FIG. **18** shows a synthetic cationic polypeptide binding to Vitroskin©, a synthetic tissue analogue. A substantial portion of applied FITC-labeled copolypeptides were shown to

remain associated with Vitroskin©, as demonstrated using 1% solutions of FITC-K_{100}(rac-L)$_{20}$ and FITC-$K_{100}L_{40}$. By comparison, the majority of labeled BSA was removed by washing. The % remaining was determined using FITC fluorescence (λ_{exc}=495 nm, λ_{em}=521 nm) after 1-10 washes and calculated as 100% minus the percentage removed.

FIG. **19** shows in vitro antimicrobial activity of K_{100}(rac-L)$_{20}$ and $K_{100}L_{40}$ (100-3000 μg/mL) on a Vitroskin© surface against *S. aureus* after 0 and 5 washes (5×1 mL). Each 3 cm×3 cm piece of Vitroskin© was incubated with *S. aureus* for 60 min.

FIG. **20** shows A) firmness values for mixtures of 1% solutions of synthetic cationic polypeptides with 1% hydroxyethyl cellulose (HEC, Natrosol HHX) in water; and B) work of adhesion values for mixtures of 1% solutions of copolypeptides with 1% hydroxyethyl cellulose (HEC, Natrosol HHX) in water.

FIG. **21** is a table (Table 5) that shows synthetic cationic polypeptide texture analysis profile data of pure polypeptides and HEC mixtures. a. Values for 1% (w/w) polypeptide in water. b. Values for 1% polypeptide/1% HEC (w/w) in water. c. Interaction parameter: $\Delta F=F_{mix(poly/HEC)}-(F_{poly}+F_{HEC})$. Where $F_{mix(poly/HEC)}$=firmness of 1% polypeptide/1% HEC, F_{poly}=firmness of 1% polypeptide solution, and F_{HEC}=firmness of 1% HEC (Natrosol HHX) in water. Similar treatment of data was performed for the work of adhesion interaction parameter.

FIG. **22** shows A) Strain and B) frequency sweep of synergistic mixture of 1% $K_{100}L_{40}$/1% HEC and individual components at 1% in water. □=1% $K_{100}L_{40}$/1% HEC, ●=1% $K_{100}L_{40}$, and ▼=1% HEC. G' values=filled symbols and G"=open symbols.

FIG. **23** demonstrates that synthetic cationic polypeptide solutions (anti-infective solutions; left) and synthetic cationic polypeptide hydrogels (barrier gel; right) are effective at coating open wounds in a porcine model. Synthetic cationic polypeptides were fluorescently-labeled.

FIG. **24** shows the antimicrobial activity of K_{100}(rac-L)$_{20}$ in a rodent infection model. Polypropylene mesh was inserted subcutaneously in rats followed by 10^7 MRSA (33593). After 15 min., either 10, 2 or 0.4 mg/mL of K_{100}(rac-L)$_{20}$ or water was added. After 2 days, implanted mesh and surrounding tissue were analyzed for MRSA bacterial counts.

FIG. **25** shows the antimicrobial activity of K_{100}(rac-L)$_{20}$ in an open-wound porcine model. Each wound was contaminated with bacteria (*S. epidermidis, P. aeruginosa* (pig clinical isolate)). After 2 hrs, wounds were rinsed with saline and treated with 5 mL of test article or water. Test articles: K_{100}(rac-L)$_{20}$ at 10, 2, 0.4 mg/mL, 10 mg/mL K_{100}(rac-L)$_{20}$ and 30 mg/mL PEG 400 in water, and deionized water as a control. Gauze soaked in test article or water was placed on top of the wounds. Wounds were biopsied at 4 hrs after treatment.

FIG. **26** shows that a $K_{100}L_{40}$ hydrogel prevents infection in an open-wound porcine model. To each wound hydrogel was applied to the wound bed and to gauze. The hydrogel-soaked gauze was placed on top of wounds. Saline was used as a control. After 15 min. each wound was contaminated with bacteria (*S. epidermidis, P. aeruginosa* (pig clinical isolate)). Hydrogel test articles: $K_{100}L_{40}$ 10 mg/mL, $K_{100}L_{40}$ 5 mg/mL and 10 mg/mL HEC, $K_{100}L_{40}$ 2 mg/mL and 15 mg/mL HEC, and saline as a control. Wounds were biopsied at 4 hrs after hydrogel application.

FIG. **27** depicts synthetic cationic polypeptides bound to a medically-acceptable sponge material. This product concept illustrates one way that copolypeptides could be brought into contact with wounds in order to facilitate the delivery of hemostatic and/or antimicrobial activity.

FIG. **28** shows the results of an in vitro whole blood clotting assay with $K_{100}L_{40}$ and a 1:1 mixture of $K_{100}L_{40}$ and HEC at 10 and 100 μg/mL. Controls were HEC alone at 10 and 100 μg/mL and a negative control of saline and a positive control of thromboplastin, TF (50 μL in 500 μL of whole blood).

FIG. **29** shows the results of a platelet aggregation assay with $K_{100}L_{40}$ and a 1:1 mixture of $K_{100}L_{40}$ and HEC at 10 and 100 μg/mL. Controls were HEC alone at 10 and 100 μg/mL, a negative control of saline, and a positive control of collagen.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In accordance with embodiments of the invention, an aqueous composition that includes a mixture of an antimicrobial cationic polypeptide and a second pharmaceutically-acceptable polymer is used in the treatment and/or prevention of infections that occur when our natural barriers of defense are broken. This novel composition can display two functions: direct antimicrobial activity and barrier activity. Embodiments of the invention addresses one or more weaknesses of previous antimicrobials described above. Notably, the inventors began with the recognition that most of the previous antiseptics and antibiotics were based on antimicrobial activity in solution with the microbes in suspension (MIC assays), a method that could lead away from effectiveness in tissues. By contrast, the inventors focused on the design and selection of agents for broad antimicrobial activity, especially at tissue surfaces and in the terrain ("cracks and crevices") of wounds. This includes the formation of a barrier containing cationic (positively charged) elements that can inhibit the movement of certain substances or cells that display anionic elements (e.g., microbes). In one embodiment, the synthetic cationic polypeptides of these compositions contain at least one cationic segment and at least one hydrophobic segment, are comprised substantially of natural amino acids, and are broadly antimicrobial (i.e., against gram positive and gram negative bacteria). They can also be designed to self-assemble, based in part on the interaction of their hydrophobic segments. Further, synthetic polypeptides are formulated with a second pharmaceutically-accepted polymer to provide a composition that is directly antimicrobial and that effectively coats tissues. These mixtures may also display hemostatic properties. In some embodiments, the second pharmaceutically acceptable polymer is not a polyethylene glycol (PEG).

Embodiments of the invention may be used alone or in combination with other materials that provide similar or complementary activities.

Synthetic Cationic Polypeptides.

A variety of synthetic cationic polypeptides can be used in the aqueous compositions described herein. In an embodiment, the synthetic cationic polypeptide comprises a segment of recurring units or residues of positively charged amino acids (e.g. lysine, arginine) and another segment of recurring units or residues of hydrophobic amino acids (e.g. leucine, isoleucine, valine, alanine). Examples include copolypeptides composed of a segment or block of one or more recurring lysine amino acids and a segment or block of one or more recurring leucine amino acids with the overall structure of K_xL_y (FIG. **1**). In various embodiments, one or more of the synthetic cationic polypeptide(s) comprise a segment having a chain length of at least 40 amino acid

residues. In various embodiments, the synthetic cationic polypeptide(s) comprises substantially all natural amino acid subunits. In some embodiments, the synthetic cationic polypeptide(s) is characterized by at least one segment containing at least five consecutive cationic amino acid residues and at least one segment containing at least five consecutive hydrophobic amino acid residues. For example, in some cases, the polypeptides include one or more segments comprising at least 5 consecutive recurring lysine amino acid units and one or more segments comprising at least 5 consecutive hydrophobic recurring amino acids (e.g., leucine). In some cases, cationic polypeptides may be relatively long-chain molecules having lysine blocks containing 50 to 200 or more lysine amino acid residues. Examples of synthetic cationic polypeptides include K_{50}(rac-L)$_{10}$, K_{50}(rac-L)$_{20}$, K_{50}(rac-L)$_{30}$, K_{50}(rac-L)$_{40}$, K_{50}(rac-L)$_{50}$, $Ka_{50}L_{10}$, $K_{50}L_{20}$, $K_{50}L_{30}$, $K_{50}L_{40}$, $K_{50}L_{50}$, K_{100}(rac-L)$_{10}$, K_{100}(rac-L)$_{20}$, K_{100}(rac-L)$_{30}$, K_{100}(rac-L)$_{40}$, K_{100}(rac-L)$_{50}$, $K_{100}L_{10}$, $K_{100}L_{20}$, $K_{100}L_{30}$, $K_{100}L_{40}$, $K_{100}L_{50}$, K_{200}(rac-L)$_{10}$, K_{200}(rac-L)$_{20}$, K_{200}(rac-L)$_{30}$, K_{200}(rac-L)$_{40}$, K_{200}(rac-L)$_{50}$, $K_{200}L_{10}$, $K_{200}L_{20}$, $K_{200}L_{30}$, $K_{200}L_{40}$, $K_{200}L_{50}$ (FIG. **2** (Table 1)). Other synthetic cationic polypeptides (e.g. with longer or shorter lysine segments and longer or shorter leucine segments) are also envisioned. Also, this invention includes other synthetic cationic polypeptides where the cationic segments can include other amino acids, such as arginine, and hydrophobic blocks that can contain one or more hydrophobic amino acids, including but not limited to leucine, alanine, valine, and/or isoleucine. In some cases, one or more of the segments may have a random sequence of amino acids, including hydrophobic and hydrophilic amino acids.

Examples of synthetic cationic polypeptides include those described in U.S. Pat. Nos. 6,680,365; 6,632,922; 6,686,446; 6,818,732; 7,329,727; US Published Patent Application No. 2008/0125581; and US Published Patent Application No. 2011/048869. The aforementioned patents and patent applications are hereby incorporated herein by reference, and particularly for the purpose of describing synthetic cationic polypeptides and method of making them.

Methods of manufacturing synthetic cationic polypeptides that have exceptionally narrow polydispersities with high reproducibility have been developed, and such polymers can be made to various specifications to suit the needs of the antimicrobial activity and the barrier formation. For example, by combining high quality α-amino acid N-carboxy anhydrides (NCAs) in anhydrous solvents with a benzyl amine initiator, a high quality block copolypeptide is synthesized. These polymers then undergo deprotection and purification to yield the final product.

Several analytical techniques have been developed and refined to both monitor the synthesis of the peptides and to analyze the resulting polymeric products for size, properties and residual impurities. Infrared spectroscopy may be used to monitor the progress of the reaction, while Size Exclusion Gel Permeation Chromatography may be used to monitor the growth and status of the polymer at various stages of the process. Other analytical techniques such as nuclear magnetic resonance spectroscopy (NMR), matrix assisted laser desorption ionization mass spectroscopy (MALDI-MS), and inductively coupled plasma mass spectroscopy (ICP-MS) may be used, e.g., as additional quality control tests to ensure consistent reproducibility and purity (FIG. **3-8**).

Synthetic cationic polypeptides can be designed to demonstrate broad-based antimicrobial activity (see US Published Patent Application No. 2011/048869). A synthetic cationic polypeptide is considered to have antimicrobial activity if it provides greater than 3 logs killing of *Staphylococcus epidermidis* and *Escherichia coli* in a standard 60 minute time-kill assays at a synthetic cationic polypeptide concentration of 1.0 mg/mL or less. In an embodiment, the synthetic cationic polypeptide has an antimicrobial activity that provides greater than 3 logs killing of *Staphylococcus epidermidis* and *Escherichia coli* in a standard 60 minute time-kill assays at a synthetic cationic polypeptide concentration of 100 μg/mL or less. As shown in FIGS. **9-12**, embodiments of these synthetic cationic polypeptides demonstrate antimicrobial activity in vitro against both Gram-positive and Gram-negative bacteria. This activity is demonstrated in in vitro time-kill assays with the synthetic cationic polypeptides in aqueous media. Further, embodiments of synthetic cationic polypeptides demonstrated anti-biofilm activity in vitro, as depicted in FIG. **13**. It is likely that these effects can be explained in part by the cationic polypeptides binding to the anionic charges present in the matrix of the biofilm. In addition, the surfactant-like activity of the synthetic cationic polypeptides may contribute to the anti-biofilm effect. It is also recognized that the inventive compositions may change the structure and/or charge of biofilms, allowing other agents (e.g., locally applied or systemically applied antibiotics) to penetrate the biofilm, thereby enhancing activity.

Mixtures of synthetic cationic polypeptides and other polymers can retain antimicrobial activity in vitro. The second pharmaceutically-acceptable polymer is different from the one or more synthetic, cationic polypeptide(s) having antimicrobial activity. In an embodiment, the second pharmaceutically-acceptable polymer has little or no antimicrobial activity itself. For example, in an embodiment, the antimicrobial activity of second pharmaceutically-acceptable polymer is less than 10% of the antimicrobial activity of the synthetic, cationic polypeptide(s).

The individual amounts of the synthetic, cationic polypeptide(s) and the second pharmaceutically-acceptable polymer in the aqueous composition are at least about 100 μg/mL, and can be higher, e.g. about 1 mg/mL, about 5 mg/mL, or about 10 mg/mL, or about 20/ml, or about 40 mg/ml or higher. The amount of the second pharmaceutically-acceptable polymer in the aqueous composition is at least about 10% by weight, based on the weight of the one or more synthetic, cationic polypeptide(s), and may be higher, e.g., at least about 20% by weight, at least about 30% by weight, or at least about 50% by weight, same basis. The synthetic, cationic polypeptide(s) and the second pharmaceutically-acceptable polymer in the aqueous composition are selected such the polymers are mutually miscible. As noted above, the synthetic, cationic polypeptide(s) with antimicrobial activity and the second pharmaceutically-acceptable polymer are considered mutually miscible if at least about 90% of the polymeric components remain mutually soluble 24 hours after mixing and maintaining at room temperature in water at a concentration of each polymer of 1 mg/mL, upon visible examination. Surprisingly, such mutual miscibility of the water, synthetic, cationic polypeptide(s) and the second pharmaceutically-acceptable polymer can be achieved, despite the expectation of phase separation due to the typical mutual incompatibility of polymers in aqueous solution at the 1 mg/rnL concentrations and molecular weights described herein. The aqueous compositions described herein can be prepared by intermixing the individual polymeric components with water, e.g., at room

temperature with stirring, using ordinary mixing methods known to those skilled in the art.

EXAMPLE 1

As depicted in FIGS. **14-16**, embodiments of synthetic cationic polypeptides were shown to retain substantially all of their antimicrobial activity in the presence of other pharmaceutically-acceptable polymers, as demonstrated by in vitro time-kill assays against *S. aureus, S. epidermidis, P. aeruginosa*, and *E. coli*. In these studies, a variety of cellulose-based second polymers were evaluated. As shown in FIGS. **11** and **13**, other studies demonstrated retained antimicrobial activity in in vitro the presence of other pharmaceutically-acceptable (polymeric surfactants). In an embodiment, the antimicrobial activity of the aqueous composition is greater than 3 logs killing of *Staphylococcus epidermidis* and *Escherichia coli* in standard 60 minute time-kill assays at a synthetic cationic polypeptide(s) concentration of 100 μg/mL or less. In another embodiment, the aqueous composition is further characterized by the ability to disrupt or inhibit a biofilm in vitro at a total polymer concentration of 40 mg/ml or less.

Mixtures of synthetic cationic polypeptides and other polymers can exhibit enhanced physical and viscoelastic properties in vitro. In developing compositions for use in patients, the need to maintain antimicrobial activity while enhancing volume, tissue coverage areas, and/or biocompatibility has been recognized. Various synthetic cationic polypeptides have been combined with other pharmaceutically-acceptable polymers in a way to retain at least about 80% (e.g., at least 90%) of the antimicrobial activity of the synthetic cationic polypeptides and, in preferred embodiments, also enhance tissue coverage. It is believed that the amphiphilic nature of the synthetic cationic polypeptides and their self-assembly in aqueous solution induce stable phase-separated (collapsed or solvated) dispersions of hydrophobic and hydrated hydrophilic material. In some embodiments, it is believed that the chain length of the copolypeptides (e.g., degree of polymerization or n>50) provides a network of partially collapsed pockets of hydrophobicity that slow down solute and solvent diffusion. The barrier nature of the materials is a product of the ability of the materials to trap and slow down water mobility, and by consequence dramatically slow down the passage of any solute or particle present in water. The length of the polypeptide strongly influences the barrier properties (e.g., by drastically reduced diffusion). Therefore, selection of a second polymer for mixing with the synthetic cationic peptide(s) should be undertaken carefully to avoid disruption of influential biophysical parameters and to achieve mutual solubility in the aqueous composition. Those skilled in the art can use routine experimentation guided by the teachings provided herein to select the polymeric components and amounts to form the aqueous compositions described herein.

The properties of the aqueous compositions described herein can be tuned by controlling the ratio of the amount of the cationic polypeptide to the second pharmaceutically-acceptable polymer(s). Non-limiting examples of these second polymers may include celluloses (e.g, hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxymethylcellulose (HMC)), alginates, collagens, polymeric surfactants, polyethylene glycols, polyvinyl alcohols, polyurethanes, polyvinyl pyrolidinones (PVP), fibrin(ogen), or blood or tissue proteins. In some embodiments, the second pharmaceutically acceptable polymer is not a polyethylene glycol (PEG). It is believed that the barrier of the composition is in dynamic equilibrium with its individual components, at least one of which is also antimicrobial. The leaching of aggregates may be an advantage because the shed material may increase the effective surface area of the barrier which may increase the effectiveness of the interaction between microbes and the barrier material (FIG. **17-19**). Barrier properties of the materials can be further enhanced by the synergistic effect of polar biopolymers that further crosslink the self-assembled co-polypeptide matrix. By formulating with different biopolymers the flow and mechanical characteristics of the materials, as well as the strength of the barrier can be adjusted and controlled.

EXAMPLE 2

A texture analysis profile has been used to determine the effects of block copolypeptide composition and hydrophobic enantiopurity on the mechanical properties of the block copolypeptide/HEC mixtures (FIG. **20**). Measurement of firmness values for individual components indicated that a 1 (w/w) solution of $K_{100}L_{40}$ in water had a firmness of 2.94+/−0.25 mN and a solution of 1% (w/w) HEC in water demonstrated a firmness of 5.38+/−0.32 mN (FIG. **21** (Table 5)). Mixing 1% (w/w) $K_{100}L_{40}$ and 1% HEC (w/w) together resulted in a substantial increase in firmness, to a value of 22.77+/−0.90 mN. This corresponds to an interaction parameter value of 14.45 mN and an overall increase of over 170% over what would be expected from the additive contributions of the individual components. As shown in FIG. **20***b*, a similar trend was observed for adhesiveness. By comparison, the combination of the diblock copolypeptide $K_{100}(rac\text{-}L)_{40}$ (containing a racemic hydrophobic block) at 1% (w/w) and HEC at 1% (w/w) concentration also demonstrated enhanced firmness and adhesiveness; however, the increase was not as pronounced as with $K_{100}L_{40}$, containing an enantiopure hydrophobic block. Hydrophobic block length was also shown to be influential. Mixtures of 1% (w/w) $K_{100}L_{20}$ and 1% (w/w) HEC showed increased firmness over either biopolymer alone, but the effect was smaller than that of $K_{100}L_{40}$. Interestingly, $K_{100}L_{20}$ was antagonistic for work of adhesion. Further, $K_{100}(rac\text{-}L)_{20}$, with its shorter racemic hydrophobic block, showed no effect. The lysine homopolypeptides, K_{100} and K_{200}, also failed to enhance the firmness or adhesiveness of HEC alone, and in fact, appeared to demonstrate antagonistic activity.

EXAMPLE 3

Rheological measurements further support the synergistic interactions between $K_{100}4$) and HEC. In FIG. **22**, effects are seen in both the oscillatory strain sweep and frequency dependent sweep. By itself, 1% (w/w) $K_{100}L_{40}$ showed characteristics of a relatively brittle, weak gel. In strain sweep analysis, the brittle nature of the gel was observed by the breakdown of the gel network at low strain rates around γ=0.01; and frequency sweep analysis indicated the formation of a weak gel with an elastic modulus (G'=22 Pa at 1 rad/s). By comparison, 1% (w/w) HEC showed rheological properties more characteristic of a viscous fluid. Strain sweep analysis of a 1% HEC solution demonstrated a higher loss modulus (G") than elastic modulus (G') throughout all strain rates tested (FIG. **21**A) and the frequency sweep also showed higher G" over G' values until higher frequencies (ca. 100 rad/s) were reached (FIG. **21**B). Substantial changes in rheological properties were observed upon mixing 1% (w/w) $K_{100}L_{40}$ with 1% (w/w) HEC. Notably, strain sweep

analysis of the mixture showed an extension of the linear viscoelastic region by an order of magnitude showing a decrease in G' around γ=0.1. The frequency sweep showed a large, synergistic increase in the elastic modulus G'=141 Pa at 1 rad/s, which is a seven-fold increase over the elastic modulus of 1% (w/w) $K_{100}L_{40}$ alone (G'=22 Pa).

The amounts and types of polymeric components in the aqueous compositions described herein can be selected to achieve various properties. In an embodiment, the aqueous composition is characterized by a barrier activity, as measured by a decrease in the diffusion rate of an anionic dye of more than 2 logs at a total polymer concentration of 40 mg/mL or less. In an embodiment, the aqueous composition is characterized by a storage modulus of at least 50 Pa at a total polymer concentration of less than 40 mg/mL. In an embodiment, the aqueous composition is characterized by a storage modulus of at least 50 Pa at a total polymer concentration of less than 40 mg/mL and an ability to pass through a 20 g needle using less than 60 N pressure. In an embodiment, the aqueous composition is characterized by an ability to pass through a 20 g needle and recover a minimum of 70% of its strength as measured by storage modulus within 10 minutes. Those skilled in the art can use routine experimentation guided by the teachings provided herein to select the polymeric components and amounts to form aqueous compositions having the properties described herein.

EXAMPLE 4

Mixtures of synthetic cationic polypeptides and other polymers can exhibit enhanced antimicrobial activity in vivo. Notably, synthetic cationic polypeptides, as micellar solutions and as hydrogels, can be used to coat tissues in vivo (FIG. **23**). This tissue coating may involve the binding of the cationic peptides, through charge interactions, to anionic charges displayed on damaged tissues (as well as anionic charges in bacterial biofilms). In addition, self-assembly and cross-linking may increase the amount of material that binds and coats a tissue (e.g. thinner or thicker coating) and therefore influence a variety of biological activities and responses.

As depicted in FIG. **24**, these synthetic cationic polypeptides are antimicrobial when locally applied in vivo. In seeking to develop improved products for in vivo applications, it has been recognized that tissue binding and tissue coating properties of these materials may substantially affect their activities, including their antimicrobial properties, their anti-biofilm properties, and their effects on tissue adhesion formation and tissue remodeling. Molecular characteristics (e.g. length, charge, etc.) of the synthetic cationic polypeptides, as well as structures that they form in aqueous environments (e.g. micelles, sheets, fibrils, hydrogels) may affect tissue binding and tissue coating. It has also been recognized that mixing these synthetic cationic polypeptides with other polymers to form aqueous compositions as described herein can alter, in various ways, their biophysical properties in aqueous media and on tissues. Therefore, we tested mixtures of synthetic cationic polypeptides and other polymers in vivo.

EXAMPLE 5

Aqueous compositions that include mixtures of synthetic cationic polypeptides with two different polymers (polyethylene glycol 400 and hydroxyethyl cellulose) were both found to be effective in vivo. As depicted in FIG. **25**, $K_{100}(rac\text{-}L)_{20}$ was effective in an porcine open-wound treatment model alone and in combination with PEG 400. As depicted in FIG. **26**, $K_{100}L_{40}$ was effective at preventing microbial contamination alone and in combination with hydroxyethyl cellulose. Further, the data suggests that enhanced biophysical properties of the mixtures can improve antimicrobial activity in vivo over the synthetic cationic copolypeptides alone. The aqueous compositions described herein can be used for any one or more treatments and/or applications, including but not limited to prevention of infections, treatment of infections, treatment for topical anti-infection, treatment for microbial decolonization, wound treatment, surgical site treatment, trauma treatment, burn treatment, treatment of diabetic foot ulcers, eye treatment, treatment of vaginal infections, treatment of urinary tract infections, hand sanitization, for coating prosthetic devices and/or implants, food preservation and solution preservation.

The aqueous compositions described herein may be formulated as solutions, emulsions, particles, or hydrogels with a variety of viscoelastic properties to enhance their antimicrobial properties, their barrier properties, or both. In one embodiment, an aqueous composition as described herein comprises a wound wash product with a single lysine-leucine block copolypeptide in water, saline, or other aqueous media that is mixed with a second polymer, typically a surfactant such as poloxamer 407. In one embodiment, an aqueous composition as described herein may comprise a viscous fluid/flowing gel that can be applied through a sprayer to coat various tissues. This could be used in open or laparoscopic approaches. These materials may by themselves or in combination with other materials be formed into a variety of dressings or bandages. These may include constituting or coating a variety of materials such as gauze or sponges. An example would include an aqueous composition as described herein (e.g., containing a synthetic block copolypeptide KxLy) in the form of a coating on gauze or alginate bandages. Another example would include a two-layer material where an aqueous composition as described herein (e.g., containing a synthetic block copolypeptide KxLy alone or with another polymer such as a collagen) coats a face of a relatively inert sponge material (e.g. polyacrylate, polyurethane, or polyhema) (FIG. **27**). In this case the embodiment could have the appearance of a rectangular sponge with one coated surface or of a spherical sponge where the entire surface is coated (reminiscent of a tennis ball). This may be particularly advantageous in the treatment of bleeding wounds where hemostasis is required (FIG. **28-29**).

An embodiment provides a method for the prevention and/or treatment of infections that includes contacting a tissue of a subject with an aqueous composition as described herein, e.g., to a wound. Another embodiment further includes applying negative-pressure to the treated wound. The subject can be an animal, preferably a human. In an embodiment, the subject is further treated systemically with an antibiotic and/or locally with another antimicrobial, and/or at least one selected from the group consisting of an antibiotic, an anti-biofilm agent, a surfactant, and a combination thereof.

The aqueous compositions described herein can further include one or more of an active pharmaceutical ingredient (API). Examples of such APIs include steroids, pro-inflammatory agents, anti-inflammatory agents, anti-acne agents, preservatives, hemostatic agents, angiogenic agents, wound healing agents, anti-cancer agents and other antimicrobial agents.

What is claimed is:

1. An aqueous composition for the prevention, inhibition, or treatment of infection comprising:

a mixture comprising one or more synthetic, cationic polypeptide(s) with antimicrobial activity; and

a second pharmaceutically-acceptable polymer that is not a synthetic, cationic polypeptide(s);

wherein the amounts of the one or more synthetic, cationic polypeptide(s) and the second pharmaceutically-acceptable polymer are each at least about 100 μg/mL based on the total volume of the aqueous composition;

wherein the amount of the second pharmaceutically-acceptable polymer is at least about 10% by weight, based on the weight of the one or more synthetic, cationic polypeptide(s);

wherein one or more of the synthetic cationic polypeptide (s) comprise a segment having a chain length of at least 40 amino acid residues; and

wherein the synthetic, cationic polypeptide(s) and the second pharmaceutically-acceptable polymer are mutually miscible in water.

2. The composition of claim 1, wherein the synthetic cationic polypeptide(s) comprises substantially all natural amino acid subunits.

3. The composition of claim 1, wherein the synthetic cationic polypeptide(s) is characterized by at least one segment containing at least five consecutive cationic amino acid residues and at least one segment containing at least five consecutive hydrophobic amino acid residues.

4. The composition of claim 1, wherein the second pharmaceutically-acceptable polymer is selected from the group consisting of cellulose, alginate, collagen, polymeric surfactant, polyethylene glycol, polyvinyl alcohol, polyurethane, polyvinyl pyrolidinone (PVP), fibrin(ogen), blood proteins and tissue proteins.

5. The composition of claim 1, wherein the antimicrobial activity is greater than 3 logs killing of *Staphylococcus epidermidis*and *Escherichia coli*in standard 60 minute time-kill assays at a synthetic cationic polypeptide(s) concentration of 100 μg/mL or less.

6. The composition of claim 1, wherein the composition is further characterized by the ability to disrupt or inhibit a biofilm in vitro at a total polymer concentration of 40 mg/ml or less.

7. The composition of claim 1, wherein the composition is further characterized by a barrier activity, as measured by a decrease in the diffusion rate of an anionic dye of more than 2 logs at a total polymer concentration of 40 mg/mL or less.

8. The composition of claim 1 wherein the composition is further characterized by a storage modulus of at least 50 Pa at a total polymer concentration of less than 40 mg/mL.

9. The composition of claim 1, wherein the composition is further characterized by an ability to pass through a 20 g needle using less than 60 N pressure.

10. The composition of claim 1, wherein the composition is further characterized by an ability to pass through a 20 g needle and recover a minimum of 70% of its strength as measured by storage modulus within 10 minutes.

11. The composition of claim 1, in the form of a solution, a gel, a cream, a foam, or a dressing.

12. The composition of claim 1, wherein the composition is further characterized as being in combination with, or binding to, a dressing material, including but not limited to a gauze or sponge.

13. The composition of claim 1, wherein the composition has pro-coagulant activity, pro-hemostatic activity, or both.

14. The composition of claim 1, further comprising an active pharmaceutical ingredient (API) selected from the group consisting of steroid, pro-inflammatory agent, anti-inflammatory agent, anti-acne agent, preservatives, hemostatic agent, angiogenic agent, wound healing agent, anti-cancer agent and other antimicrobial agent.

15. The use of the composition as of claim 1 for any one or more selected from the group consisting of prevention of infection, treatment of infections, treatment for topical anti-infection, treatment for microbial decolonization, wound treatment, surgical site treatment, trauma treatment, burn treatment, treatment of diabetic foot ulcers, eye treatment, treatment of vaginal infections, treatment of urinary tract infections, hand sanitization, for coating prosthetic devices and/or implants, food preservation and solution preservation comprising applying the composition to a surface on which antimicrobial activity is desired.

16. A method for the prevention and/or treatment of infections comprising: contacting a tissue of a subject with the composition of claim 1.

17. The method of claim 16, further comprising applying negative-pressure to a wound.

18. The method of claim 16, further comprising: treating the subject systemically with other antibiotics and/or locally with another antimicrobial, and/or at least one selected from the group consisting of an antibiotic, an anti-biofilm agent, a surfactant, and a combination thereof.

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